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(54) **NOVEL SERINE-THREONINE KINASE GENE**

(57) A novel gene having the consensus sequence of a serine-threonine kinase active site has been isolated by the suppression subtractive hybridization method which comprises preparing a library of genes expressed specifically in fetal livers and isolating clones from this library at random. This gene presumably participates in cell growth control because it is highly expressed, especially in actively growing cells, and exhibits a significant homology with a vaccinia virus B1R kinase gene. Thus, it can be utilized as a target for developing cell growth inhibitors or antitumor agents.

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## Description

## Technical Field

5 [0001] The present invention relates to a novel serine-threonine kinase gene.

## Background Art

10 [0002] Fetal tissues are comprised of many undifferentiated cells that proliferate actively, highly activated cells, nascent vascular endothelial cells, and so on. Although the activity of these cells in fetal tissues is stringently regulated and inhibited as individuals mature, the state of fetal tissues can be considered similar to that of a solid tumor except that the activity is regulated. Therefore, some of the genes expressed specifically or more strongly in fetal tissues (fetal genes) can be genes involved in the phenomena characteristic of solid tumors such as abnormal growth, immortalization, infiltration, metastasis, and angiogenesis. In addition, some diseases other than tumors are also supposed to arise because fetal genes, which are repressed in a normal living body, are abnormally activated. Therefore, genes involved in various diseases such as tumors can be screened by isolating and analyzing fetal genes.

15 [0003] However, there are still few reports on systematic analysis focusing merely on fetal genes from these viewpoints, and at present there is a far from perfect understanding of these gene groups.

## 20 Disclosure of the Invention

[0004] An objective of this invention is to isolate genes expressed specifically in fetal tissues and to screen genes related to diseases.

25 [0005] The present inventors thought that fetal tissue cells could be a model for solid tumor cells and that genes involved in diseases such as tumors could be screened by isolating and analyzing fetal genes. Furthermore, the present inventors thought it possible to develop a medicine with a novel action mechanism by designing drugs targeting the genes. Based on these thoughts, the present inventors have tried to isolate fetal genes.

30 [0006] Specifically, the present inventors prepared a subtraction library with many genes expressed specifically in fetal livers (or more strongly than adult livers) by the suppression subtractive hybridization method, extracted clones from this library at random, and analyzed their structure. By doing so, the present inventors succeeded in isolating a novel gene, VRK1, having the consensus sequence of a serine-threonine kinase active site. The present inventors also performed a data base search based on the amino acid sequence deduced from the isolated gene. The present inventors thus have found this gene product exhibits a significant homology with B1R kinase, which is presumably involved in DNA replication of vaccinia virus. In addition, the present inventors found human EST having a very high homology with this gene in the database and isolated its full-length cDNA, VRK2. Analyzing the expression of the two isolated genes in various cells by northern blot analysis showed that these genes are strongly expressed, especially in actively growing cells such as human fetal livers, testes, and various tumor cell lines. Furthermore, the present inventors have found that the VRK1 protein actually has protein kinase activity.

35 [0007] Thus, the present invention relates to novel serine-threonine kinase genus, VRK1 and VRK2. More specifically, the present invention relate to:

- (1) a protein having the amino acid sequence of SEQ ID NO: 2, or a protein having the same amino acid sequence where one or more amino acids are added, deleted, or substituted and having serine-threonine kinase activity,
- 45 (2) a protein having the amino acid sequence of SEQ ID NO: 4, or a protein having the same amino acid sequence where one or more amino acids are added, deleted, or substituted and having serine-threonine kinase activity,
- (3) a protein encoded by a DNA sequence that hybridizes with the DNA sequence of SEQ ID NO: 1 or its complementary sequence and having serine-threonine kinase activity,
- (4) a protein encoded by a DNA sequence that hybridizes with the DNA sequence of SEQ ID NO: 3 or its complementary sequence and having serine-threonine kinase activity,
- 50 (5) a DNA encoding the protein of any one of (1) to (4),
- (6) a vector comprising the DNA of (5),
- (7) a transformant carrying the vector of (6),
- (8) a method of producing the protein of any one of (1) to (4), wherein the method comprises cultivating the transformant of (7),
- 55 (9) an antibody binding to the protein of any one of (1) to (4),
- (10) an antisense DNA against the DNA of (5) or part of it,
- (11) a method of screening compounds having inhibitory activity of serine-threonine kinase activity of the protein of any one of (1) to (4), wherein the method comprises

- (a) contacting the protein of any one of (1) to (4) with a substrate to be phosphorylated by this protein in the presence of a test compound to detect the kinase activity of the protein of any one of (1) to (4), and  
 (b) comparing the kinase activity detected in step (a) with that detected in the absence of the test compound and selecting a compound that lowers the kinase activity of the protein of any one of (1) to (4).

**[0008]** The present invention relates to novel serine-threonine kinases, "VRK1" and "VRK2." The nucleotide sequence of the "VRK1" cDNA and the amino acid sequence of the protein are shown in SEQ ID NO: 1 and 2, respectively. In addition, the nucleotide sequence of the "VRK2" cDNA and the amino acid sequence of the protein are shown in SEQ ID NO: 3 and 4, respectively. "VRK1" cDNA has a significant homology with B1R kinase, which is presumably involved in DNA replication of vaccinia virus. The gene is also characterized by its strong expression in actively growing cells such as fetal livers, testes, and various tumor cell lines. In addition, overexpression of "VRK1" protein drastically increases the growing activity of NIH3T3 cells. These facts imply "VRK1" is involved in the regulation mechanism of cell growth. "VRK1" protein has protein kinase activity, which presumably plays an important role in the regulation of cell growth. "VRK2" has a high homology with "VRK1," especially in the serine-threonine kinase site. "VRK2," like "VRK1," has a significant homology with B1R kinase, and the gene is characterized by its strong expression in actively growing cells such as fetal livers, testes, and various tumor cell lines. These facts imply "VRK2" has the same function as that of "VRK1."

**[0009]** "VRK1" and "VRK2" proteins can be prepared as recombinant proteins with recombinant DNA techniques or as natural proteins. The recombinant proteins can be prepared, for example, by cultivating cells transformed with the DNAs encoding these proteins, as will be described later. Natural proteins can be isolated from fetal livers, testes, or tumor cell strains such as HeLa S3, in which these proteins are highly expressed, by a method well-known to one skilled in the art, such as affinity chromatography with the antibodies of the present invention as described later. Either polyclonal or monoclonal antibodies can be used. The polyclonal antibodies can be prepared from, for example, serum from small animals such as rabbits immunized with these proteins by, for example, ammonium sulfate precipitation, protein A- or protein G-column chromatography, DEAE ion exchange chromatography, affinity chromatography using a column coupled with these proteins, etc. The monoclonal antibodies can be prepared as follows. First, a small animal such as a mouse is immunized with these proteins. The spleen is extracted from the mouse and dissociated to cells. The resulting cells are fused to mouse myeloma cells using a reagent such as polyethylene glycol, and the clone that produces antibodies against these proteins is screened from the fusion cells (hybridoma) thus generated. The hybridoma thus obtained is then transplanted into a mouse abdominal cavity. Ascites is collected from the mouse and purified by, for example, ammonium sulfate precipitation, protein A- or protein G-column chromatography, DEAE ion exchange chromatography, affinity chromatography using a column coupled with "VRK1" or "VRK2" protein, etc. If the antibodies obtained are to be used for administering to a human body (for antibody therapy or the like), humanized antibodies or human antibodies should be used to decrease immunogenicity. An example of methods for humanizing antibodies is the CDR graft method, in which an antibody gene is cloned from monoclonal antibody-producing cells and its antigenic determinant is transplanted to an existing human antibody. Besides, human antibodies can be directly prepared just like usual monoclonal antibodies by immunizing a mouse whose immune system is replaced with a human immune system.

**[0010]** Furthermore, one skilled in the art can prepare not only natural "VRK1" and "VRK2" proteins (SEQ ID NO: 2 and 4, respectively) but also proteins with substantially the same function as that of the natural proteins, if needed, by replacing amino acids in the proteins by a well-known method. Besides, mutations of amino acids in proteins can occur naturally. Thus, mutant proteins with serine-threonine kinase activity that are generated by introducing amino acid substitution, deletion, or addition into the natural proteins are also included in the proteins of the present invention. Methods for amino acid alteration, for example, a site-directed mutagenesis system using PCR (GIBCO-BRL, Gaithersburg, Maryland), the oligonucleotide-mediated site-directed mutagenesis method (Kramer, W. and Fritz, HJ (1987) Methods in Enzymol. 154:350-367), and the Kunkel method (Methods Enzymol. 85, 2763-2766 (1988)), are well-known to one skilled in the art. Furthermore, usually ten or less, preferably six or less, and more preferably three or less amino acids are substituted. The site where substitution, deletion, or addition is introduced is not particularly limited as long as the serine-threonine kinase activity is maintained. From the viewpoint of protein activity, the addition, deletion, or substitution of amino acids should be performed in a region other than the region corresponding to the consensus sequence of a serine-threonine kinase active site and to the consensus sequence of a protein kinase ATP binding site. Moreover, serine-threonine kinase activity of a protein can be detected, for example, by the method described in Example 9, mentioned later.

**[0011]** Furthermore, one skilled in the art can usually isolate DNAs having a high homology with the DNA encoding "VRK1" or "VRK2" protein (SEQ ID NO: 1 or 3, respectively) based on the DNA or the part of it using a hybridization technique (Sambrook, J. et al., Molecular Cloning 2nd ed. 9.47-9.58, Cold Spring Harbor Lab. Press, 1989) and obtain proteins having substantially the same function as VRK1 or VRK2 protein (SEQ ID NO: 2 or 4, respectively) from the DNA. Thus, proteins with serine-threonine kinase activity that are encoded by DNAs hybridizing with DNA encoding "VRK1" or "VRK2" protein are also included in the proteins of the present invention. Hybridizing DNAs are isolated from

other organisms including, for example, mice, rats, rabbits, bovines, and so on. Tissues such as fetal livers and testes are especially suitable for isolating. Thus isolated DNAs encoding proteins having substantially the same function as that of "VRK1" or "VRK2" proteins usually have a high homology with the DNA (SEQ ID NO: 1 or 3) encoding "VRK1" or "VRK2" protein, respectively. The term "high homology" used herein means at least 40% or more, preferably 60% or more, and more preferably 80% or more of sequence identity at the amino acid level. From the viewpoint of the protein activity, a high homology should be found in the regions corresponding to the consensus sequence of a serine-threonine kinase active site and to the consensus sequence of a protein kinase ATP binding site.

**[0012]** Examples of conditions for hybridization to isolate these DNAs are as follows. After prehybridization at 55°C for 30 minutes or longer, hybridization is performed by adding labeled probes and incubating at 37 to 55°C for an hour or longer using "ExpressHyb Hybridization Solution" (CLONTECH). After that, the resulting hybridized product is washed three times for 20 minutes each at room temperature in 2 x SSC and 0.1% SDS then once at 37°C in 1 x SSC and 0.1% SDS. More preferably, after prehybridization at 60°C for 30 minutes or longer, hybridization is performed by adding labeled probes and incubating at 60°C for an hour or longer using "ExpressHyb Hybridization Solution" (CLONTECH). Thereafter, the hybridized product is washed three times for 20 minutes each at room temperature in 2 x SSC and 0.1% SDS then twice at 50°C in 0.1 x SSC and 0.1% SDS. Still more preferably, after prehybridization at 68°C for 30 minutes or longer, hybridization is performed by adding labeled probes and incubating at 68 °C for an hour or longer using "ExpressHyb Hybridization Solution" (CLONTECH). Thereafter, the hybridized product is washed three times for 20 minutes each at room temperature in 2 x SSC and 0.1% SDS then twice at 50°C in 0.1 x SSC and 0.1% SDS.

**[0013]** The present invention also relates to the DNAs encoding the above-described proteins of the present invention. The DNAs of the present invention include CDNAs, genomic DNAs, and synthetic DNAs as long as they encode the proteins of the present invention. The DNAs of the present invention can be used to produce the recombinant proteins. Specifically, the recombinant proteins can be prepared by inserting the DNA (for example, the DNA of SEQ ID NO: 1 or 3) of the present invention into a suitable expression vector, cultivating the transformant obtained by introducing the vector into suitable cells, and purifying the expressed proteins. For example, mammalian cells such as COS, CHO, or NIH3T3 cells; insect cells such as Sf9 cells; yeast cells; and *E. coli* cells can be used for producing the recombinant proteins. Vectors for expressing recombinant proteins in these cells vary depending on the host cells. For example, pcDNA3 (Invitrogen) or pEF-BOS (Nucleic Acids. Res. 1990, 18(17), p5322) is used for mammalian cells; "BAC-to-BAC baculovirus expression system" (GIBCO BRL), for insect cells; "Pichia Expression Kit" (Invitrogen), for yeast cells; and pGEX-5X-1 (Pharmacia) or "QIAexpress system" (Qiagen), for *E. coli* cells. Vectors can be introduced into host cells by, for example, the method using calcium phosphate, DEAE dextran, or cationic liposome DOTAP (Boehringer Mannheim); electroporation; the calcium chloride method; etc. The recombinant proteins can be purified from the obtained transformants by the usual methods such as the method described in "The QIAexpressionist handbook, Qiagen, Hilden, Germany."

**[0014]** Furthermore, the DNAs of the present invention can be used for gene therapy of diseases caused by mutations in genomic DNAs. In gene therapy, the DNAs of the present invention are administered to a living body inserted into adenovirus vectors (e.g., pAdexLcw), retrovirus vectors (e.g., pZIPneo) and so on. They can be administered by either *ex vivo* methods or *in vivo* methods.

**[0015]** Furthermore, since the proteins of the present invention are presumably involved in the regulation of cell growth, antisense DNAs against the DNAs of the present invention or part of them can be used as inhibitors for developing cell growth or as antitumor agents. The antisense DNAs are administered to a living body directly or in the form of the vectors into which they have been inserted. The antisense DNAs can be synthesized by methods well known to one skilled in the art.

**[0016]** The present invention also relates to a method of screening compounds having inhibitory activity of serine-threonine kinase activity of the proteins of the present invention. This screening method comprises two steps. First, the protein of the present invention is caused to contact a substrate to be phosphorylated by this protein in the presence of a test compound to detect the kinase activity of the protein of the present invention. Second, the kinase activity detected in step (a) is compared with that detected in the absence of the test compound, and a compound that lowers the kinase activity of the protein of the present invention is selected.

**[0017]** Test compounds used for this screening method are not particularly limited and are generally low-molecular-weight compounds, proteins (including the above-described antibodies of the present invention), peptides, etc. Test compounds are either artificially synthesized or natural. Substrates are, for example, casein, I $\kappa$ B $\alpha$  protein, etc. The kinase activity of the protein of the present invention can be detected, for example, by adding ATP having radioactively labeled phosphate to the reaction system containing the protein of the present invention and the substrate and measuring the radioactivity of the phosphate attached to the substrate. Specifically, the activity is detected by the method described in Example 9. The compounds thus isolated can be used as cell growth inhibitors or antitumor agents. Moreover, the present inventors learned that "VRK1" protein phosphorylates I $\kappa$ B $\alpha$  protein. I $\kappa$ B $\alpha$  is thought to be rapidly degraded when phosphorylated, thereby releasing and activating NF- $\kappa$ B bound thereto. In addition, NF- $\kappa$ B is well known as a central transcriptional regulator that causes widespread immune reactions and inflammation reactions.

Therefore, compounds that inhibit the kinase activity of the proteins of the present invention can be used as antiplo-  
gistics and immunosuppressants.

#### Brief Description of the Drawings

[0018]

Figure 1 shows the adapters used for constructing the subtraction library.

Figure 2A shows the consensus sequence of the active site of serine-threonine kinase. Figure 2B shows the con-  
sensus sequence of the ATP binding site of protein kinase.

Figure 3 shows the nucleotide sequence of the clone fls223 and its deduced amino acid sequences.

Figure 4 shows electrophoretic patterns demonstrating the result of RT-PCR analysis performed to detect expres-  
sions of VRK1 and VRK2 genes in fetal and adult livers. In the figure, "A" and "F" represent Adult liver, and Fetal  
liver. "Low," "Middle," and "High" represent the level of PCR cycles.

Figure 5 compares amino acid sequences of VRK1 and B1R.

Figure 6 compares amino acid sequences of VRK1 and VRK2.

Figure 7 compares amino acid sequences of VRK2 and B1R.

Figure 8 shows electrophoretic patterns demonstrating the result of northern blot analysis of the expression of  
VRK1 and VRK2 genes in various cells.

Figure 9 shows an electrophoretic pattern demonstrating the result of western blot analysis using anti c-Myc anti-  
body. Cell extracts from COS7 cells transfected with plasmid DNA, pcDNA3 (lane 1) or pcDNA3/VRK1myc (lane 2),  
were examined.

Figure 10 shows an electrophoretic pattern demonstrating the result of northern blot analysis using the VRK1  
cDNA as a probe. Total RNA samples prepared from NIH3T3 cells transfected with plasmid DNA, pCOS (lane 1)  
or pCOS/VRK1w (lane 2), and from a human hepatoma cell line, HepG2 cells (lane 3), were examined.

Figure 11 presents microscopic photographs showing the result of colony assay. A pool of NIH3T3 cells transfected  
with plasmid DNA, pCOS ("pCOS") or pCOS/VRK1w ("pCOS/VRK1w") was examined.

Figure 12 shows an electrophoretic pattern of purified GST fusion proteins (CBB staining). Fusion proteins with wild  
VRK1 protein (lane 1) or with a mutant one (lane 2) were examined.

Figure 13 shows electrophoretic patterns demonstrating the result of kinase assay. Added proteins are indicated by  
"+" on the upper portion. Arrows indicate phosphorylated GST-VRK1 ("A," autophosphorylation), phosphorylated  
casein ("C"), phosphorylated GST-IkB $\alpha$  ("I"), and phosphorylated IkB $\alpha$  C-terminal peptide ("P").

Figure 14 shows an electrophoretic pattern demonstrating the result of kinase assay. Reactions were performed in  
the presence of various divalent cations at various concentrations as indicated on the right. Arrows indicate phos-  
phorylated GST-VRK1 ("A," autophosphorylation), and phosphorylated casein ("C").

Figure 15 shows an electrophoretic pattern demonstrating the result of western blot analysis with an antibody  
against a VRK1 peptide using K562 cell extracts.

#### Best Mode for Implementing the Invention

[0019] The present invention is illustrated below in detail with reference to examples, but is not to be construed as  
being limited thereto.

#### Example 1

##### Construction of a subtraction library

[0020] A subtraction library was prepared using the PCR-Select™ cDNA Subtraction kit (CLONTECH) basically  
according to the method described by Luda Diatchenko et al. (Proc. Natl. Acad. Sci. USA, Vol.93, 6025-6030, 1996).

[0021] First, double-stranded cDNAs were synthesized from polyA<sup>+</sup> RNA prepared from human fetal and adult livers  
by the standard method using MMLV reverse transcriptase. Next, the respective cDNAs were blunt-ended with T4 DNA  
polymerase, then cleaved by *Rsa*I. A part of the cDNA originating from fetal liver (tester) was split in two; one of which  
was ligated with the adapter-1 and the other with the adapter-2 (Figure 1). Each aliquot was mixed with an excess  
amount of the adult liver cDNA (driver), denatured by heat, and subjected to the first hybridization at 68°C for 8 hours.  
Aliquots were then combined without heat denaturation, mixed further with an excess amount of heat-denatured driver,  
and subjected to the second hybridization at 68°C for about 16 hours. The mixture was diluted in the dilution buffer,  
incubated at 75°C for 7 minutes to remove the shorter strands of adapters, and used as a template for PCR. By per-  
forming PCR with primers corresponding to the adapters, "PCR primer-1" (SEQ ID NO: 5) and "PCR primer-2" (SEQ

ID NO: 6), cDNAs carrying different adapters on their two ends (subtracted cDNAs) were selectively amplified (suppression PCR). To obtain products with further selectivity, a portion of the amplified products was used as a template for PCR with primers "Nested PCR primer-1" (SEQ ID NO: 7) and "Nested PCR primer-2" (SEQ ID NO: 8), which locate further inside of the primers; "PCR primer-1" (SEQ ID NO: 5); and "PCR primer-2" (SEQ ID NO: 6). The products were purified using the "QIAquick PCR Purification kit" (QIAGEN), and cloned into the pT7Blue-T vector (Novagen) by the TA cloning method to create a subtraction library.

### Example 2

#### Sequence analysis

[0022] Plasmid DNA prepared by the alkali SDS method or products of colony PCR were used as a template for sequence reaction. Sequence reaction was performed by the cycle-sequencing method using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit With AmplyTaq DNA Polymerase, FS, and the result was analyzed by the ABI 377 DNA Sequencer.

[0023] Colony PCR was performed as follows. Colonies carrying recombinant vectors were directly suspended into PCR reaction mixtures that contain vector primers, "M13 P4-22 primer" (SEQ ID NO: 9) and "M13 P5-22 primer" (SEQ ID NO: 10). After PCR reaction, amplified insert DNA was separated from unreacted primers and nucleotides by gel filtration or the like, and used as a template for sequencing.

[0024] As a result, the clone fls223 (261 bp) (later renamed "VRK1") was found to be able to encode an amino acid sequence (Figure 3) that contains the consensus sequence of the active site of serine-threonine kinase ([Leu, Ile, Val, Met, Phe, Tyr, Cys]-Xaa-[His, Tyr]-Xaa-Asp-[Leu, Ile, Val, Met, Phe, Tyr]-Lys-Xaa-Xaa-Asn-[Leu, Ile, Val, Met, Phe, Tyr, Cys, Thr]-[Leu, Ile, Val, Met, Phe, Tyr, Cys, Thr]-[Leu, Ile, Val, Met, Phe, Tyr, Cys, Thr]) (corresponds to amino acids at 173-185 of SEQ ID NO: 2) (Figure 2A). In addition, no gene registered in the database completely matches the nucleotide sequence of this clone. Thus, the gene is a novel one.

### Example 3

#### RT-PCR assay

[0025] Using polyA<sup>+</sup> RNA extracted from fetal and adult livers, single-stranded cDNAs were synthesized by the standard method with SUPERScript™ II RNase H<sup>-</sup> Reverse Transcriptase (GIBCO BRL). Some of the cDNAs were used as a template for RT-PCR analysis of fls223. PCR was performed using TaKaRa Taq (TaKaRa) as Taq polymerase by the hot-start method, where the reaction was started by adding TaqStart™ Antibody (CLONTECH). Primers "FLS223 S1 primer" (SEQ ID NO: 11) and "FLS223 A1 primer" (SEQ ID NO: 12) were used to amplify fls223.

[0026] The G3PDH (glyceraldehyde 3-phosphate dehydrogenase) gene, which is a housekeeping gene equally expressed in various tissues and known to be influenced only slightly by various inducers on its expression, was used as a control. G3PDH was amplified using the primers "hG3PDH5' primer" (SEQ ID NO: 13) and "hG3PDH3' primer" (SEQ ID NO: 14). RT-PCR analysis confirmed that the clone fls223 is strongly expressed in fetal liver, and its expression was also detected in adult liver (Figure 4). The full-length cDNA was then cloned for more detailed analysis of the gene.

### Example 4

#### Cloning by Rapid Amplification of cDNA End (RACE)

[0027] The Marathon™ Ready cDNA (CLONTECH) or cDNA prepared by the Marathon™ cDNA Amplification Kit (CLONTECH) was used as a template for 5' RACE and 3' RACE (Chenchik A. et al., CLONTECHniques X, 1, 5-8, 1995).

[0028] The primers described above, "FLS223 S1 primer" (SEQ ID NO: 11) and "FLS223 A1 primer" (SEQ ID NO: 12), were used for 5' RACE and 3' RACE of VRK1/fls223. Using a combination of these primers and a primer AP1 (SEQ ID NO: 15), corresponding to the adapter of the template cDNA, the reaction performed basically consisted of a reaction at 94°C for 2 minutes; five cycles of reactions at 94°C for 30 seconds and at 68°C 4 minutes; and 30 cycles of reactions at 94°C for 30 seconds, 62°C for 1 minute, and 72°C for 3 minutes; and a reaction at 72°C for 10 minutes. TaKaRa Ex Taq (TaKaRa) was used for PCR, and the reaction was started by the hot-start method by adding TaqStart™ Antibody (CLONTECH). After reaction, detected bands were recovered using the QIAquick Gel Extraction Kit (QIAGEN), and subcloned into the pT7Blue-T vector (Novagen).

[0029] Analysis of the entire nucleotide sequence revealed that the full-length fls223 cDNA encodes an open reading frame composed of 396 amino acids (refer to SEQ ID NO: 1). In the former part of the amino acid sequence, there exists

a consensus sequence of the ATP binding site of protein kinase ([Leu, Ile, Val]-Gly-Xaa-Gly-Xaa-[Phe, Tyr, Trp, Met, Gly, Ser, Thr, Asn, His]-[Ser, Gly, Ala]-Xaa-[Leu, Ile, Val, Cys, Ala, Thr]-Xaa-Xaa-[Gly, Ser, Thr, Ala, Cys, Leu, Ile, Val, Met, Phe, Tyr]-Xaa(five times or 18 times)-[Leu, Ile, Val, Met, Phe, Tyr, Trp, Cys, Ser, Thr, Ala, Arg]-[Ala, Ile, Val, Pro]-[Leu, Ile, Val, Met, Phe, Ala, Gly, Cys, Lys, Arg]-Lys) (corresponds to the amino acids 43-71 described in the SEQ ID NO: 2) (Figure 2B), and a consensus sequence of the active site of serine-threonine kinase, which is also found in the original clone. Thus, the gene product is assumed to be a novel serine-threonine kinase.

[0030] A homology search of the whole database revealed that the gene shows high homology to the B1R gene product of the Vaccinia virus (J. Gen. Virol., 70, 3187-3201, 1989; J. Gen. Virol., 72, 1349-1376, 1991) (Figure 5). The B1R gene encodes a protein composed of 300 amino acids and is assumed to be a serine-threonine kinase because the gene contains the consensus sequences analogous to that of the ATP binding site of protein kinase and of the active site of serine-threonine kinase. The full-length fls223 cDNA and the B1R gene showed relatively high homology over the entire region as well as in the kinase domain (Smallest Sum probability in Blast search =  $2.7e-78$ ). Therefore, the gene is named "Vaccinia virus B1R kinase related Kinase 1" (VRK1).

[0031] B1R kinase is an early gene whose expression is observed in early stages. It appears several hours after vaccinia virus infection and is then repressed. It has been shown that in a mutant strain containing a point mutation on the gene, virus replication stops during DNA replication. Thus, it has been hypothesized that B1R kinase regulates virus DNA replication (J. Biol. Chem., 264, 21458-21461, 1989).

[0032] VRK1 also exhibits an obvious homology to B1R kinase in the region outside of the serine-threonine kinase domain. Thus, VRK1 may participate in the regulation of cellular DNA replication or, more widely, in cell growth control, as is the case for B1R kinase in virus. This notion is supported by the fact that the VRK1 genes are more strongly expressed in tissues such as fetal liver and the testis, where numerous actively growing cells exist.

[0033] Furthermore, a public clone "human EST - H80169," which has an extremely high homology to VRK1, was found by searching the data base. Using the primers "RK A2 primer" (SEQ ID NO: 16) and "RK S1 primer" (SEQ ID NO: 17) for 5' RACE and 3' RACE, the full-length cDNA of the gene was cloned as described for VRK1, and the entire nucleotide sequence was determined. As a result, it was found that the gene encodes an open reading frame composed of 508 amino acids (refer to SEQ ID NO: 3), in which the consensus sequence of the active site of serine-threonine kinase exists. Thus, this gene may also encode a novel serine-threonine kinase. The amino acid sequence has an extremely high homology to VRK1, especially near the kinase domain (Figure 6), and a high homology to the vaccinia virus B1R kinase (Figure 7). These suggest a close relationship between this kinase and B1R kinase. Thus, it was named "Vaccinia virus B1R kinase related Kinase 2" (VRK2).

[0034] RT-PCR confirmed that VRK2 is also expressed more strongly in fetal liver than in adult liver (Figure 4). The primers "RK S2 primer" (SEQ ID NO: 18) and "RK A2 primer" (SEQ ID NO: 16) were used for RT-PCR.

### Example 5

#### Chromosome mapping

[0035] Chromosome mapping of the VRK1 and VRK2 genes was performed using the GENEBRIDGE 4 Radiation Hybrid Panel (Research Genetics, Inc.) (Nature Genetics, 7, 22-28, 1994). DNA on the panel was used as a template for PCR. For VRK1, PCR was performed with a combination of the above primers ("FLS223 S1 primer" (SEQ ID NO: 9) and "FLS223 A1 primer" (SEQ ID NO: 12)) by a reaction at 94°C for 5 minutes; five cycles of reactions at 94°C for 30 seconds and at 72°C for 2 minutes; and 30 cycles of reactions at 94°C for 30 seconds and at 68°C for 2 minutes; and a reaction at 72°C for 3 minutes. For VRK2, PCR was performed with a combination of primers "VRK2 A primer" (SEQ ID NO: 19) and "VRK2 B primer" (SEQ ID NO: 20) by a reaction at 94°C for 3 minutes; 30 cycles of reactions at 94°C for 30 seconds, 60°C for 1 minute, and 72°C for 2 minutes; and a reaction at 72°C for 5 minutes. The resulting pattern was analyzed on a database on Internet (<http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>), and maps were obtained.

[0036] The VRK1 gene was thus mapped to the position between the STS markers "D14S265" and "APM063XE7" on chromosome 14. Similarly, the VRK2 gene was mapped to the position between the STS markers "CHLC.GATA23H01" and "D2S357" on chromosome 2.

### Example 6

#### Northern blot analysis

[0037] The expressions of VRK1 and VRK2 mRNA in various human normal tissues and tumor cell lines were analyzed by northern blotting (Figure 8).

[0038] The 5' terminal fragment of the VRK1 cDNA (upstream region of the HindIII site at nucleotide residue 546) or



that of the VRK2 cDNA (upstream region of the *EcoRI* site at position 426) was labeled with [ $\alpha$ - $^{32}$ P]dCTP by the random primer method using Ready-to-Go DNA labeling beads (Pharmacia), and used as a probe. Hybridization was performed at 68°C in ExpressHyb Hybridization Solution (CLONTECH) using Multiple Tissue Northern (MTN) Blot - Human, Human II, Human Fetal II, and Human Cell line (CLONTECH) according to the method recommended by the manufacturer. Final wash was done at 50°C in 0.1 x SSC, 0.1% SDS, and the image on the filter processed through hybridization was analyzed with the BAS-2000II bioimaging analyzer (Fuji Photo Film).

[0039] In Figure 8, tumor cell lines used are malignant melanoma cells "G361," lung carcinoma cells "A549," colorectal adenocarcinoma cells "SW480," Burkitt's lymphoma cells "Raji," acute lymphoblastic leukemia cells (T cell) "MOLT-4," chronic myelogenous leukemia cells "K-562," uterocervical carcinoma cells "HeLaS3," and promyelocytic leukemia cells "HL60".

[0040] The results revealed that VRK1 was expressed relatively highly in fetal tissues, and extremely highly in fetal liver. VRK1 was expressed weakly in almost all adult tissues, but it was expressed strongly in the testis and the thymus. In tumor cell lines, a very strong expression of VRK1 was observed in six out of eight cell lines.

[0041] The expression pattern at VRK2 was basically similar to that of VRK1; VRK2 was expressed strongly in fetal liver and the testis. Similarly, it was expressed strongly in tumor cell lines. However, VRK2 was not expressed in MOLT-4 cells, in contrast to the pattern of VRK1 mRNA.

### Example 7

#### Constructing expression plasmid DNAs

[0042] cDNA containing the entire coding region of VRK1 or VRK2 was amplified by PCR using a combination of primers, VRK1 S1 primer (SEQ ID NO: 21) and VRK1 A1 primer (SEQ ID NO: 22), or VRK2 S1 primer (SEQ ID NO: 23) and VRK2 A1 primer (SEQ ID NO: 24) from cDNA synthesized from polyA<sup>+</sup> RNA extracted from human fetal liver. The amplified product was cleaved at the *NotI* site that is attached to the end of the primers and purified by agarose gel electrophoresis to obtain DNA fragments of the correct size. These were subcloned into the pCOS vector, which was pre-treated with *NotI* and dephosphorylated on its ends with alkaline-phosphatase/CIAP (TaKaRa). This vector contains an EF1 $\alpha$  promoter and enables expressing cloned cDNA strongly in a broad range of mammalian cell lines. By sequencing the thus-obtained subclones, clones (pCOS/VRK1w, pCOS/VRK2w) without mutation such as PCR error were selected and used for expression as described below and for further construction of expression plasmid DNA.

[0043] Plasmids for expressing proteins in which the anti c-Myc antibody epitope sequence (SEQ ID NO: 25) is attached to the C-terminus were constructed as follows. Using about 50 nanograms of the plasmid DNA and with pCOS/VRK1w or pCOS/VRK2w as a template, PCR was performed with a combination of primers. These included VRK1 MYC1 primer (SEQ ID NO: 26) and VRK1 MYC2 primer (SEQ ID NO: 27), or VRK2 MYC1 primer (SEQ ID NO: 28) and VRK2 MYC2 primer (SEQ ID NO: 29), and cDNA with the anti c-Myc antibody epitope attached to the C-terminus of the coding sequence was amplified. KOD DNA polymerase (TOYOBO) was used as the DNA polymerase. The amplified product was cleaved at the *BamHI* site that is attached to the end of the primers and purified by agarose gel electrophoresis to obtain DNA fragments of the correct size. These were subcloned into the pcDNAS vector (Invitrogen), which was digested with *BamHI* and dephosphorylated on its ends with alkaline-phosphatase/CIAP (TaKaRa). By sequencing the thus-obtained subclones, clones (pcDNA3/VRK1myc, pcDNA3/VRK2myc) without mutation such as PCR error were selected, and used for later experiments.

[0044] Expression plasmid DNAs for glutathione-S-transferase (GST) fusion proteins in *E. coli* were constructed as follows. Using the plasmid DNA pCOS/VRK1w or pCOS/VRK2w as a template, the coding region was amplified by PCR with a combination of primers, VRK1 H3 primer (SEQ ID NO: 30) and VRK1 H4 primer (SEQ ID NO: 31), or VRK2 H3 primer (SEQ ID NO: 32) and VRK2 H4 primer (SEQ ID NO: 33). The amplified product was cleaved at the *BamHI* site that is attached to the end of the primers purified by agarose gel electrophoresis to obtain DNA fragments of the correct size. These were then subcloned into the pGEX-5X-1 vector (Pharmacia), which was digested with *BamHI* and dephosphorylated on its ends with alkaline-phosphatase/CIAP (TaKaRa). By sequencing the thus-obtained subclones, clones (pGEX/VRK1w, pGEX/VRK2w) without mutation such as PCR error were selected and used for later experiments.

[0045] A clone with a mutation introduced to the predicted ATP binding site within the kinase catalytic domain (Lys at position 71 in the amino acid sequence of SEQ ID NO: 2 is replaced by Trp) was constructed using the Chameleon™ Double-Stranded Site-Directed Mutagenesis Kit (STRATAGENE) as follows. About one microgram of the pGEX/VRK1w plasmid DNA was mixed with primers VRK1 KW1 primer (SEQ ID NO: 34) and a selection primer, Select1 primer (SEQ ID NO: 35), and heat denatured by boiling for 5 minutes. Plasmid DNA and both primers containing mutation were then annealed by incubating at room temperature for 30 minutes. Next, new DNA strands were synthesized from primers by adding substrate nucleotides, DNA polymerase, etc. These were treated with *PstI* to digest wild plasmid DNA, and introduced into XLmutS competent cells. After overnight liquid culture, plasmid DNA was extracted then treated with *PstI* to digest contaminating wild plasmid DNA. It was then reintroduced into the competent cells. By sequencing sev-



eral single isolated colonies, a clone (pGEX/VRK1K71W) with an introduced mutation was selected.

### Example 8

#### 5 Expression in mammalian cell lines

[0046] About 10 micrograms of plasmid DNA, pcDNA3/VRK1myc or pcDNA3, was introduced (transfected) into COS7 cells using SuperFect (QIAGEN). Specifically, about  $10^6$  COS7 cells were plated in a 10-cm dish, and cultured overnight. A mixture of 10 micrograms of plasmid DNA and 60 microliters of SuperFect was then added to the culture, and the culture was incubated for about 3 hours. Thereafter, the culture medium was replaced with fresh medium. The cells were then cultured for two more days and collected by detaching in a trypsin-EDTA solution. Cells were washed once in PBS, disrupted in RIPA buffer (1% NP-40, 10 mM Tris-HCl (pH 7.2), 0.1% sodium deoxycholate, 0.1% SDS, 0.15 M sodium chloride, 1 mM EDTA, 10 micrograms/ml aprotinin, 1 mM PMSF), and cell extracts were obtained by centrifugation. The cell extracts were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and subjected to western blot analysis using anti c-Myc antibody (SANTA CRUZ). A band of about 50 kDa was specifically observed in cells transfected with the pcDNA3/VRK1myc plasmid DNA, indicating that VRK1myc protein is expressed (Figure 9).

[0047] Next, about 7.5 micrograms of plasmid DNA, pCOS/VRK1w or pCOS, was transfected into NIH3T3 cells by the method using cationic phospholipid DOTAP (Boehringer Mannheim). After transfection, transformants were selected by adding G418 (GIBCO-BRL) to the culture medium to a final concentration of 500 micrograms/ml. Total RNA was prepared from each pool of transformants by the method using ISOGEN (Wako Junyaku). The total RNA was then subjected to northern blot analysis using the VRK1 cDNA as a probe. The results confirmed that VRK1 mRNA was expressed in a pool of cells obtained by transfection with the pCOS/VRK1w plasmid DNA (Figure 10). These pools of cells were examined for the ability to form colonies on soft agar (colony assay). To this end,  $2 \times 10^4$  cells were suspended in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and 0.4% thawed Sea-Plaque agarose (TaKaRa), and overlaid on a bottom agarose which was made of 0.53% SeaPlaque agarose, 10% fetal bovine serum, and DMEM. After two-week culturing, the pool of cells obtained by transfection with the pCOS/VRK1w plasmid DNA formed a number of colonies that were obviously larger than those formed in the pool of cells obtained by transfection with the pCOS plasmid DNA. This suggests that overexpression of VRK1 confers abnormal growth activities on cells (Figure 11).

### Example 9

#### Expression of VRK1 protein in *E.coli* and kinase assay

[0048] Both wild VRK1 protein and mutant VRK1 protein were expressed in *E.coli* as a fusion protein with GST protein and purified. The *E.coli* DH5 $\alpha$  strain cells carrying the above-described plasmid DNA, pGEX/VRK1W, or pGEX/VRK1K71W were cultured overnight at 37°C in 10 ml 2xYT medium. Some of the culture was diluted 100-fold with fresh 2xYT medium and cultured at 37°C until the OD value at 600 nm reached 0.6. IPTG (isopropyl- $\beta$ -D(-)-thiogalactopyranoside) was then added to the culture to a final concentration of 0.1 mM, and the culture was incubated further for several hours. The *E. coli* cells were collected by centrifugation, resuspended in PBS containing 1% Triton X-100 and 1% Tween 20, and subsequently disrupted by sonication to solubilize proteins. From the solubilized samples, wild VRK1 protein and mutant VRK1, which were expressed as a fusion protein with GST, were purified by affinity chromatography using glutathione Sepharose4B (Pharmacia). These proteins were subjected to SDS-PAGE and stained with Coomassie Brilliant Blue (CBB) to confirm their purity (Figure 12). GST protein and GST-IkB $\alpha$  protein were prepared in the same manner.

[0049] Kinase assay was performed on a total of 50 microliters of a reaction mixture containing 0.2 micrograms of wild or mutant VRK1 protein, 50 mM Tris-HCl (pH 7.2), 1 mM dithiothreitol (DTT), 2 mM or 10 mM of divalent cation (Mg, Mn, Zn, Ca), a maximum of 5 micrograms of substrate protein, and 1 microliter of [ $\gamma$ - $^{32}$ P]-ATP (3000 Ci/mM, 10 mCi/ml [Amersham]). In some experiments, another buffer system containing 40 mM Hepes (pH 7.4), 1 mM DTT, and 2.5 mM EGTA was used.

[0050] Specifically, the reaction was first performed in the presence of 10 mM Mg at 37°C for 30 minutes using, as a protein substrate, histone (Nakalai), casein (Sigma), myelin basic protein/MBP (Sigma), GST, GST-IkB $\alpha$ , or IkB $\alpha$  C-terminal peptide (SEQ ID NO: 36). The reaction mixture was subjected to SDS-PAGE, and the radioactivity of phosphorylated proteins was analyzed with a BAS2000II bioimaging analyzer (Fuji Photo Film). The result indicates that wild VRK1 protein phosphorylates casein and GST-IkB $\alpha$  (Figure 13). In contrast, no phosphorylation was observed in reactions with mutant VRK1 carrying mutation in the predicted ATP binding site. This indicates that VRK1 is a protein kinase that contains a typical catalytic domain. In addition, GST protein was not phosphorylated by VRK1, suggesting that the phosphorylation of GST-IkB $\alpha$  protein by VRK1 occurs within IkB $\alpha$  protein but not in GST moiety.

[0051] I $\kappa$ B $\alpha$  is said to negatively regulate the function of transcription factor NF- $\kappa$ B by forming a complex with it. In addition, it is widely accepted that I $\kappa$ B $\alpha$  is inactivated by self-phosphorylation, immediately thereafter undergoes proteolysis, thereby liberating and activating NF- $\kappa$ B. NF- $\kappa$ B is supposed to be a central transcriptional regulator that induces a broad range of immune reactions and inflammatory reactions. Therefore, a kinase that phosphorylates I $\kappa$ B $\alpha$  is important as a target molecule of anti-inflammatory drugs. Since VRK1 strongly phosphorylates I $\kappa$ B $\alpha$  *in vitro*, VRK1 probably participates in the activation of NF- $\kappa$ B by phosphorylating I $\kappa$ B $\alpha$  *in vivo* as well. Therefore, it is possible to anticipate anti-inflammatory effects or immunosuppressive effects by inhibiting VRK1 kinase activity, or by reducing its protein amount.

[0052] Next, the requirement for divalent cations in phosphorylation by VRK1 was examined (Figure 14). In the presence of various divalent cations (Mg, Mn, Zn, and Ca) at a final concentration of 2 mM or 10 mM, kinase reactions were performed using casein as a substrate protein. The result showed that VRK1 exhibits phosphorylation activity in the presence of all divalent cations except for Zn. However, the levels of activity were different; VRK1 exhibited especially strong activity in the presence of Mn.

#### Example 10

##### Preparation of an antibody against VRK1 protein

[0053] A peptide (SEQ ID NO: 37) corresponding to the C-terminal sequence of the deduced VRK1 amino acid sequence was synthesized (Sawady Technology) and conjugated to Keyhole limpet hemocyanin (KLH) at its amino-terminal cysteine mediated by m-maleimidobenzoyl-N-hydroxy-succinimide ester (MBS). This was used as an antigen to immunize rabbits, and antisera were obtained. Antibodies that specifically react with the peptide were purified from the antisera by affinity chromatography using Cellulofine (Seikagaku Corporation) conjugated with the peptide. Western blot analysis using extracts of K562 cells, which were confirmed by northern blot analysis to strongly express VRK1, detected a single band with a molecular weight of approximately 50 kDa, indicating that VRK1 protein is specifically recognized by the antibody (Figure 15).

#### Industrial Applicability

[0054] The serine-threonine kinase genes isolated by the present inventors show significant homology to a vaccinia virus gene that is thought to be involved in DNA replication and are strongly expressed in actively growing cells. Furthermore, overexpression of proteins encoded by the genes remarkably enhances cell proliferation activity. Thus, the isolated serine-threonine kinase genes are assumed to participate in the regulation of cell growth. Therefore, it is possible to develop cell growth inhibitors or antitumor agents based on a novel mechanism by screening drugs targeted on the genes (such as antisense DNA), or drugs which can regulate either the expression of the genes or the activity of the proteins encoded by the genes of the present invention.

Sequence Listing

(1) Name of Applicant: Chugai Research Institute for Molecular Medicine,  
Inc.

(2) Title of the Invention: Novel Serine-threonine Kinase Gene

(3) Reference Number: C1-805PCT

(4) Application Number:

(5) Filing Date:

(6) Country where the priority application was filed and the application  
number of the priority application: Japan, No. Hei 8-357864

(7) Priority date: December 26, 1996

(8) Number of Sequences: 37

SEQ ID NO: 1

SEQUENCE LENGTH: 1662

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

FEATURE:

NAME/KEY: CDS

LOCATION: 76..1266

SEQUENCE DESCRIPTION:

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GCGGCTTAGG TGAAA ATG CCT CGT GTA AAA GCA GCT CAA GCT GGA AGA CAG 111

Met Pro Arg Val Lys Ala Ala Gln Ala Gly Arg Gln

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	1	5	10	
5	AGC TCT GCA AAG ACA CAT CTT GCA GAA CAA TTT GCA GTT GGA GAG ATA	159		
	Ser Ser Ala Lys Arg His Leu Ala Glu Gln Phe Ala Val Gly Glu Ile			
	15	20	25	
10	ATA ACT GAC ATG GCA AAA AAG GAA TGG AAA GTA GGA TTA CCC ATT GGC	207		
	Ile Thr Asp Met Ala Lys Lys Glu Trp Lys Val Gly Leu Pro Ile Gly			
15	30	35	40	
	CAA GGA GGC TTT GGC TGT ATA TAT CTT GCT GAT ATG AAT TCT TCA GAG	255		
	Gln Gly Gly Phe Gly Cys Ile Tyr Leu Ala Asp Met Asn Ser Ser Glu			
20	45	50	55	60
	TCA GTT GGC AGT GAT GCA CCT TGT GTT GTA AAA GTG GAA CCC AGT GAC	303		
25	Ser Val Gly Ser Asp Ala Pro Cys Val Val Lys Val Glu Pro Ser Asp			
	65	70	75	
30	AAT GGA CCT CTT TTT ACT GAA TTA AAG TTC TAC CAA CGA GCT GCA AAA	351		
	Asn Gly Pro Leu Phe Thr Glu Leu Lys Phe Tyr Gln Arg Ala Ala Lys			
	80	85	90	
35	CCA GAG CAA ATT CAG AAA TGG ATT CGT ACC CGT AAG CTG AAG TAC CTG	399		
	Pro Glu Gln Ile Gln Lys Trp Ile Arg Thr Arg Lys Leu Lys Tyr Leu			
40	95	100	105	
	GGT GTT CCT AAG TAT TGC GGG TCT GGT CTA CAT GAC AAA AAT CGA AAA	447		
	Gly Val Pro Lys Tyr Trp Gly Ser Gly Leu His Asp Lys Asn Gly Lys			
45	110	115	120	
	AGT TAC AGG TTT ATG ATA ATG GAT CGC TTT GGG AGT GAC CTT CAG AAA	495		
50	Ser Tyr Arg Phe Met Ile Met Asp Arg Phe Gly Ser Asp Leu Gln Lys			
	125	130	135	140

	ATA TAT GAA GCA AAT GCC AAA AGG TTT TCT CGG AAA ACT GTC TTG CAG	543
5	Ile Tyr Glu Ala Asn Ala Lys Arg Phe Ser Arg Lys Thr Val Leu Gln	
	145 150 155	
	CTA AGC TTA AGA ATT CTG GAT ATT CTG GAA TAT ATT CAC GAG CAT GAG	591
10	Leu Ser Leu Arg Ile Leu Asp Ile Leu Glu Tyr Ile His Glu His Glu	
	160 165 170	
15	TAT GTG CAT GGA GAT ATC AAG GCC TCA AAT CTT CTT CTG AAC TAC AAG	639
	Tyr Val His Gly Asp Ile Lys Ala Ser Asn Leu Leu Leu Asn Tyr Lys	
	175 180 185	
20	AAT CCT GAC CAG GTG TAC TTG GTA GAT TAT GGC CTT GCT TAT CGG TAC	687
	Asn Pro Asp Gln Val Tyr Leu Val Asp Tyr Gly Leu Ala Tyr Arg Tyr	
25	190 195 200	
	TGC CCA GAA GGA GTT CAT AAA GAA TAC AAA GAA GAC CCC AAA AGA TGT	735
30	Cys Pro Glu Gly Val His Lys Glu Tyr Lys Glu Asp Pro Lys Arg Cys	
	205 210 215 220	
	CAC GAT GGC ACT ATT GAA TTC ACG AGC ATC GAT GCA CAC AAT GGT GTG	783
35	His Asp Gly Thr Ile Glu Phe Thr Ser Ile Asp Ala His Asn Gly Val	
	225 230 235	
40	GCC CCA TCA AGA CGT CGT GAT TTG GAA ATA CTT GGT TAT TGC ATG ATC	831
	Ala Pro Ser Arg Arg Gly Asp Leu Glu Ile Leu Gly Tyr Cys Met Ile	
	240 245 250	
45	CAA TGG CTT ACT GGC CAT CTT CCT TGG GAG GAT AAT TTG AAA GAT CCT	879
	Gln Trp Leu Thr Gly His Leu Pro Trp Glu Asp Asn Leu Lys Asp Pro	
50	255 260 265	
	AAA TAT GTT AGA GAT TCC AAA ATT AGA TAC AGA GAA AAT ATT GCA AGT	927
55		

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Lys Tyr Val Arg Asp Ser Lys Ile Arg Tyr Arg Glu Asn Ile Ala Ser

270 275 280

TTG ATG GAC AAA TGT TTT CCT GAG AAA AAC AAA CCA GGT GAA ATT GCC 975

Leu Met Asp Lys Cys Phe Pro Glu Lys Asn Lys Pro Gly Glu Ile Ala

285 290 295 300

AAA TAC ATG GAA ACA GTG AAA TTA CTA GAC TAC ACT GAA AAA CCT CTT 1023

Lys Tyr Met Glu Thr Val Lys Leu Leu Asp Tyr Thr Glu Lys Pro Leu

305 310 315

TAT GAA AAT TTA CGT GAC ATT CTT TTG CAA GGA CTA AAA GCT ATA GGA 1071

Tyr Glu Asn Leu Arg Asp Ile Leu Leu Gln Gly Leu Lys Ala Ile Gly

320 325 330

AGT AAG GAT GAT GGC AAA TTG GAC CTC AGT GTT GTG GAG AAT GGA GGT 1119

Ser Lys Asp Asp Gly Lys Leu Asp Leu Ser Val Val Glu Asn Gly Gly

335 340 345

TTG AAA GCA AAA ACA ATA ACA AAG AAG CGA AAG AAA GAA ATT GAA GAA 1167

Leu Lys Ala Lys Thr Ile Thr Lys Lys Arg Lys Lys Glu Ile Glu Glu

350 355 360

AGC AAG GAA CCT GGT GTT GAA GAT ACG GAA TGG TCA AAC ACA CAG ACA 1215

Ser Lys Glu Pro Gly Val Glu Asp Thr Glu Trp Ser Asn Thr Gln Thr

365 370 375 380

GAG GAG GCC ATA CAG ACC CGT TCA AGA ACC AGA AAG AGA GTC CAG AAG 1263

Glu Glu Ala Ile Gln Thr Arg Ser Arg Thr Arg Lys Arg Val Gln Lys

385 390 395

TAATTCAGAT GCTGTGAACC AGATTTCCTT TTCTTTGTTT TCTTTTGAAT TTTTCTCCT 1323

TTTCTGTTAG AACTGTTTAA TTTTCCTGTC AGTCTTCCGA GGTGGAATTA ATGATTAAAT 1383

ACTCATGTGT TCAGAAAACA TAACTTTTT TTATAAAAT ATTTGTACA ATTCATTAAA 1443  
 5 GGCTAATTTA TGAAATTGA AAATCTTCAG GTTATACTCC TTAAGTTATC CCAAAGCCGT 1503  
 GTGTTTGTGA TGTTTTGGAG TACATATATA TGAAAATTAT TATGACACGC ACTTTTCTAA 1563  
 TCATTGTACA TTTCTCAGAG TGGATAAAAA TGTTTGACAA AGTCCTCACT TTTAAGGAAA 1623  
 10 TGCAAAGCTT AAAATAAAAC TCTCTTTGT TTGATGCAG 1662

15 SEQ ID NO: 2

SEQUENCE LENGTH: 396

20 SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULE TYPE: protein

25 SEQUENCE DESCRIPTION:

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1 5 10

30 Ser Ser Ala Lys Arg His Leu Ala Glu Gln Phe Ala Val Gly Glu Ile

15 20 25

Ile Thr Asp Met Ala Lys Lys Glu Trp Lys Val Gly Leu Pro Ile Gly

30 35 40

Gln Gly Gly Phe Gly Cys Ile Tyr Leu Ala Asp Met Asn Ser Ser Glu

45 50 55 60

45 Ser Val Gly Ser Asp Ala Pro Cys Val Val Lys Val Glu Pro Ser Asp

65 70 75

50 Asn Gly Pro Leu Phe Thr Glu Leu Lys Phe Tyr Gln Arg Ala Ala Lys

80 85 90



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Pro Glu Gln Il Gln Lys Trp Il Arg Thr Arg Lys Leu Lys Tyr Leu

95

100

105

Gly Val Pro Lys Tyr Trp Gly Ser Gly Leu His Asp Lys Asn Gly Lys

110

115

120

Ser Tyr Arg Phe Met Ile Met Asp Arg Phe Gly Ser Asp Leu Gln Lys

125

130

135

140

Ile Tyr Glu Ala Asn Ala Lys Arg Phe Ser Arg Lys Thr Val Leu Gln

145

150

155

Leu Ser Leu Arg Ile Leu Asp Ile Leu Glu Tyr Ile His Glu His Glu

160

165

170

Tyr Val His Gly Asp Ile Lys Ala Ser Asn Leu Leu Leu Asn Tyr Lys

175

180

185

Asn Pro Asp Gln Val Tyr Leu Val Asp Tyr Gly Leu Ala Tyr Arg Tyr

190

195

200

Cys Pro Glu Gly Val His Lys Glu Tyr Lys Glu Asp Pro Lys Arg Cys

205

210

215

220

His Asp Gly Thr Ile Glu Phe Thr Ser Ile Asp Ala His Asn Gly Val

225

230

235

Ala Pro Ser Arg Arg Gly Asp Leu Glu Ile Leu Gly Tyr Cys Met Ile

240

245

250

Gln Trp Leu Thr Gly His Leu Pro Trp Glu Asp Asn Leu Lys Asp Pro

255

260

265

Lys Tyr Val Arg Asp Ser Lys Ile Arg Tyr Arg Glu Asn Ile Ala Ser

270

275

280

Leu Met Asp Lys Cys Phe Pro Glu Lys Asn Lys Pro Gly Glu Ile Ala

285 290 295 300

Lys Tyr Met Glu Thr Val Lys Leu Leu Asp Tyr Thr Glu Lys Pro Leu

305 310 315

Tyr Glu Asn Leu Arg Asp Ile Leu Leu Gln Gly Leu Lys Ala Ile Gly

320 325 330

Ser Lys Asp Asp Gly Lys Leu Asp Leu Ser Val Val Glu Asn Gly Gly

335 340 345

Leu Lys Ala Lys Thr Ile Thr Lys Lys Arg Lys Lys Glu Ile Glu Glu

350 355 360

Ser Lys Glu Pro Gly Val Glu Asp Thr Glu Trp Ser Asn Thr Gln Thr

365 370 375 380

Glu Glu Ala Ile Gln Thr Arg Ser Arg Thr Arg Lys Arg Val Gln Lys

385 390 395

SEQ ID NO: 3

SEQUENCE LENGTH: 1833

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

FEATURE:

NAME/KEY: CDS

LOCATION: 131..1657

SEQUENCE DESCRIPTION:

CTGCACTGCG AGGCCGACGC AGCTGGAGAG AAGTTAGGCA GGTCCCTAGGG AGGCCAGGCT 60  
 5 CGACTGCTGG GCCCGCCTCC CCGCGGGACT GTAGGCCCGG GGGCTCCGCC TCGTCGCAGC 120  
 GGCAGAAGTG ATG CCA CCA AAA AGA AAT GAA AAA TAC AAA CTT CCT ATT 169

Met Pro Pro Lys Arg Asn Glu Lys Tyr Lys Leu Pro Ile

1 5 10  
 CCA TTT CCA GAA GGC AAG GTT CTG GAT GAT ATG GAA GGC AAT CAG TGG 217

15 Pro Phe Pro Glu Gly Lys Val Leu Asp Asp Met Glu Gly Asn Gln Trp

15 20 25  
 GTA CTG GGC AAG AAG ATT GGC TCT GGA GGA TTT GGA TTG ATA TAT TTA 263

20 Val Leu Gly Lys Lys Ile Gly Ser Gly Gly Phe Gly Leu Ile Tyr Leu

30 35 40 45  
 25 GCT TTC CCC ACA AAT AAA CCA GAG AAA GAT GCA AGA CAT CTA GTA AAA 313

Ala Phe Pro Thr Asn Lys Pro Glu Lys Asp Ala Arg His Val Val Lys

50 55 60  
 30 GTC GAA TAT CAA GAA AAT GGC CCG TTA TTT TCA GAA CTT AAA TTT TAT 361

Val Glu Tyr Gln Glu Asn Gly Pro Leu Phe Ser Glu Leu Lys Phe Tyr

65 70 75  
 35 CAG AGA GTT GCA AAA AAA GAC TGT ATC AAA AAG TGG ATA GAA CGC AAA 409

Gln Arg Val Ala Lys Lys Asp Cys Ile Lys Lys Trp Ile Glu Arg Lys

80 85 90  
 40 CAA CTT GAT TAT TTA GGA ATT CCT CTG TTT TAT GGA TCT GGT CTG ACT 457

Gln Leu Asp Tyr Leu Gly Ile Pro Leu Phe Tyr Gly Ser Gly Leu Thr

95 100 105  
 45 GAA TTC AAG GGA AGA AGT TAC AGA TTT ATG GTA ATG GAA AGA CTA GGA 505

Glu Phe Lys Gly Arg Ser Tyr Arg Phe Met Val Met Glu Arg Leu Gly

	110	115	120	125	
5	ATA GAT TTA CAG AAG ATC TCA GGC CAG AAT GGT ACC TTT AAA AAG TCA				553
	Ile Asp Leu Gln Lys Ile Ser Gly Gln Asn Gly Thr Phe Lys Lys Ser				
		130	135	140	
10	ACT GTC CTG CAA TTA GGT ATC CGA ATG TTG GAT GTA CTG GAA TAT ATA				601
	Thr Val Leu Gln Leu Gly Ile Arg Met Leu Asp Val Leu Glu Tyr Ile				
		145	150	155	
15	CAT GAA AAT GAA TAT GTT CAT GGT GAT GTA AAA GCA GCA AAT CTA CTT				649
	His Glu Asn Glu Tyr Val His Gly Asp Val Lys Ala Ala Asn Leu Leu				
20		160	165	170	
	TTG GGT TAC AAA AAT CCA GAC CAG GTT TAT CTT GCA GAT TAT GGA CTT				697
25	Leu Gly Tyr Lys Asn Pro Asp Gln Val Tyr Leu Ala Asp Tyr Gly Leu				
		175	180	185	
30	TCC TAC AGA TAT TGT CCC AAT GGG AAC CAC AAA CAG TAT CAG GAA AAT				745
	Ser Tyr Arg Tyr Cys Pro Asn Gly Asn His Lys Gln Tyr Gln Glu Asn				
		190	195	200	205
35	CCT AGA AAA GGC CAT AAT GGG ACA ATA GAG TTT ACC AGC TTG GAT GCC				793
	Pro Arg Lys Gly His Asn Gly Thr Ile Glu Phe Thr Ser Leu Asp Ala				
		210	215	220	
40	CAC AAG GGA CTA GCC TTG TCC AGA CGA AGT GAC GTT CAG ATC CTC GGC				841
	His Lys Gly Val Ala Leu Ser Arg Arg Ser Asp Val Glu Ile Leu Gly				
45		225	230	235	
	TAC TGC ATG CTG CGG TGG TTG TGT GGC AAA CTT CCC TGG GAA CAG AAC				889
50	Tyr Cys Met Leu Arg Trp Leu Cys Gly Lys Leu Pro Trp Glu Gln Asn				
		240	245	250	

55

EP 0 960 938 A1

CTG AAG GAC CCT GTG GCT GTG CAG ACT GCT AAA ACA AAT CTG TTG GAC 937  
5 Leu Lys Asp Pro Val Ala Val Gln Thr Ala Lys Thr Asn Leu Leu Asp  
255 260 265

GAG CTC CCC CAG TCA GTG CTT AAA TGG GCT CCT TCT GGA AGC AGT TGC 985  
10 Glu Leu Pro Gln Ser Val Leu Lys Trp Ala Pro Ser Gly Ser Ser Cys  
270 275 280 285

TGT GAA ATA GCC CAA TTT TTG GTA TGT GCT CAT AGT TTA GCA TAT GAT 1033  
15 Cys Glu Ile Ala Gln Phe Leu Val Cys Ala His Ser Leu Ala Tyr Asp  
290 295 300

GAA AAG CCA AAC TAT CAA GCC CTC AAG AAA ATT TTG AAC CCT CAT GGA 1081  
20 Glu Lys Pro Asn Tyr Gln Ala Leu Lys Lys Ile Leu Asn Pro His Gly  
305 310 315

ATA CCT TTA GGA CCA CTG GAC TTT TCC ACA AAA GGA CAG AGT ATA AAT 1129  
25 Ile Pro Leu Gly Pro Leu Asp Phe Ser Thr Lys Gly Gln Ser Ile Asn  
320 325 330

GTC CAT ACT CCA AAC AGT CAA AAA GTT GAT TCA CAA AAG GCT GCA ACA 1177  
30 Val His Thr Pro Asn Ser Gln Lys Val Asp Ser Gln Lys Ala Ala Thr  
335 340 345

AAG CAA GTC AAC AAG GCA CAC AAT AGG TTA ATC GAA AAA AAA GTC CAC 1225  
35 Lys Gln Val Asn Lys Ala His Asn Arg Leu Ile Glu Lys Lys Val His  
350 355 360 365

AGT GAG AGA AGC GCT GAG TCC TGT GCA ACA TGG AAA GTG CAG AAA GAC 1273  
40 Ser Glu Arg Ser Ala Glu Ser Cys Ala Thr Trp Lys Val Gln Lys Glu  
370 375 380

GAG AAA CTG ATT GGA TTG ATG AAC AAT GAA GCA GCT CAG GAA AGC ACA 1321  
45  
50  
55

Glu Lys Leu Ile Gly Leu Met Asn Asn Glu Ala Ala Gln Glu Ser Thr

	385	390	395	
5				
	AGG AGA AGA CAG AAA TAT CAA GAG TCT CAA GAA CCT TTG AAT GAA GTA	1369		
	Arg Arg Arg Gln Lys Tyr Gln Glu Ser Gln Glu Pro Leu Asn Glu Val			
10	400	405	410	
	AAC AGT TTC CCA CAA AAA ATC AGC TAT ACA CAA TTC CCA AAC TCA TTT	1417		
15	Asn Ser Phe Pro Gln Lys Ile Ser Tyr Thr Gln Phe Pro Asn Ser Phe			
	415	420	425	
	TAT GAG CCT CAT CAA GAT TTT ACC AGT CCA GAT ATA TTC AAG AAG TCA	1465		
20	Tyr Glu Pro His Gln Asp Phe Thr Ser Pro Asp Ile Phe Lys Lys Ser			
	430	435	440	445
25	AGA TCT CCA TCT TGG TAT AAA TAC ACT TCC ACA GTC AGC ACG GGG ATC	1513		
	Arg Ser Pro Ser Trp Tyr Lys Tyr Thr Ser Thr Val Ser Thr Gly Ile			
	450	455	460	
30	ACA GAC TTA GAA AGT TCA ACT GGA CTT TGG CCT ACA ATT TCC CAG TTT	1561		
	Thr Asp Leu Glu Ser Ser Thr Gly Leu Trp Pro Thr Ile Ser Gln Phe			
35	465	470	475	
	ACT CTT AGT GAA CAG ACA AAC GCA GAT GTT TAT TAT TAT CGC ATC ATC	1609		
40	Thr Leu Ser Glu Glu Thr Asn Ala Asp Val Tyr Tyr Tyr Arg Ile Ile			
	480	485	490	
	ATA CCT GTC CTT TTG ATG TTA GTA TTT CTT GCT TTA TTT TTT CTC	1654		
45	Ile Pro Val Leu Leu Met Leu Val Phe Leu Ala Leu Phe Phe Leu			
	495	500	505	
50	TGAACATGAT ACCAAATTC CTTTGTATAA TTTTAAAGT TTCCAGCTCT TCACCGAAAT	1714		
	GTTGTATTCT TATTTCACTG TTTCCTTCCA GACATTTTAA AGGTAATTGG CTTTAAAAAG	1774		
55				

AGAACATATT TTAACAAAGT TTGTG ACAC TCTAAAAAAT AAAATTGCTT TGTACTAGT 1833

5

SEQ ID NO: 4

SEQUENCE LENGTH: 508

10

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

15

MOLECULE TYPE: protein

SEQUENCE DESCRIPTION:

20

Met Pro Pro Lys Arg Asn Glu Lys Tyr Lys Leu Pro Ile

1 5 10

Pro Phe Pro Glu Gly Lys Val Leu Asp Asp Met Glu Gly Asn Gln Trp

25

15 20 25

Val Leu Gly Lys Lys Ile Gly Ser Gly Gly Phe Gly Leu Ile Tyr Leu

30

30 35 40 45

Ala Phe Pro Thr Asn Lys Pro Glu Lys Asp Ala Arg His Val Val Lys

35

50 55 60

Val Glu Tyr Gln Glu Asn Gly Pro Leu Phe Ser Glu Leu Lys Phe Tyr

65 70 75

40

Gln Arg Val Ala Lys Lys Asp Cys Ile Lys Lys Trp Ile Glu Arg Lys

80 85 90

Gln Leu Asp Tyr Leu Gly Ile Pro Leu Phe Tyr Gly Ser Gly Leu Thr

45

95 100 105

Glu Phe Lys Gly Arg Ser Tyr Arg Phe Met Val Met Glu Arg Leu Gly

50

110 115 120 125

Ile Asp Leu Gln Lys Ile Ser Gly Gln Asn Gly Thr Phe Lys Lys Ser

55



	130	135	140
5	Thr Val Leu Gln Leu Gly Ile Arg Met Leu Asp Val Leu Glu Tyr Ile		
	145	150	155
10	His Glu Asn Glu Tyr Val His Gly Asp Val Lys Ala Ala Asn Leu Leu		
	160	165	170
15	Leu Gly Tyr Lys Asn Pro Asp Gln Val Tyr Leu Ala Asp Tyr Gly Leu		
	175	180	185
20	Ser Tyr Arg Tyr Cys Pro Asn Gly Asn His Lys Gln Tyr Gln Glu Asn		
	190	195	200
25	Pro Arg Lys Gly His Asn Gly Thr Ile Glu Phe Thr Ser Leu Asp Ala		
	210	215	220
30	His Lys Gly Val Ala Leu Ser Arg Arg Ser Asp Val Glu Ile Leu Gly		
	225	230	235
35	Tyr Cys Met Leu Arg Trp Leu Cys Gly Lys Leu Pro Trp Glu Gln Asn		
	240	245	250
40	Leu Lys Asp Pro Val Ala Val Gln Thr Ala Lys Thr Asn Leu Leu Asp		
	255	260	265
45	Glu Leu Pro Gln Ser Val Leu Lys Trp Ala Pro Ser Gly Ser Ser Cys		
	270	275	280
50	Cys Glu Ile Ala Gln Phe Leu Val Cys Ala His Ser Leu Ala Tyr Asp		
	290	295	300
55	Glu Lys Pro Asn Tyr Gln Ala Leu Lys Lys Ile Leu Asn Pro His Gly		
	305	310	315
	Ile Pro Leu Gly Pro Leu Asp Phe Ser Thr Lys Gly Gln Ser Ile Asn		
	320	325	330

Val His Thr Pro Asn Ser Gln Lys Val Asp Ser Gln Lys Ala Ala Thr

5                    335                    340                    345

Lys Gln Val Asn Lys Ala His Asn Arg Leu Ile Glu Lys Lys Val His

10                    350                    355                    360                    365

Ser Glu Arg Ser Ala Glu Ser Cys Ala Thr Trp Lys Val Gln Lys Glu

15                    370                    375                    380

Glu Lys Leu Ile Gly Leu Met Asn Asn Glu Ala Ala Gln Glu Ser Thr

                    385                    390                    395

Arg Arg Arg Gln Lys Tyr Gln Glu Ser Gln Glu Pro Leu Asn Glu Val

20                    400                    405                    410

Asn Ser Phe Pro Gln Lys Ile Ser Tyr Thr Gln Phe Pro Asn Ser Phe

25                    415                    420                    425

Tyr Glu Pro His Gln Asp Phe Thr Ser Pro Asp Ile Phe Lys Lys Ser

30                    430                    435                    440                    445

Arg Ser Pro Ser Trp Tyr Lys Tyr Thr Ser Thr Val Ser Thr Gly Ile

35                    450                    455                    460

Thr Asp Leu Glu Ser Ser Thr Gly Leu Trp Pro Thr Ile Ser Gln Phe

                    465                    470                    475

Thr Leu Ser Glu Glu Thr Asn Ala Asp Val Tyr Tyr Tyr Arg Ile Ile

40                    480                    485                    490

Ile Pro Val Leu Leu Met Leu Val Phe Leu Ala Leu Phe Phe Leu

45                    495                    500                    505

50                    SEQ ID NO: 5

SEQUENCE LENGTH: 22

55

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

CTAATACGAC TCACTATAGG GC

22

SEQ ID NO: 6

SEQUENCE LENGTH: 21

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

TGTAGCGTGA AGACGACAGA A

21

SEQ ID NO: 7

SEQUENCE LENGTH: 21

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

TCGAGCGGCC GCCCGGCCAGG T

21

SEQ ID NO: 8

SEQUENCE LENGTH: 21

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

AGGGCGTGGT CCGGACGGCGG T

21

SEQ ID NO: 9

SEQUENCE LENGTH: 22

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

CCAGGCTTTT CCCAGTCACG AC

22

SEQ ID NO: 10

SEQUENCE LENGTH: 22

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

TCACACAGGA AACAGCTATG AC

22

5

SEQ ID NO: 11

10

SEQUENCE LENGTH: 24

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

15

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

20

SEQUENCE DESCRIPTION:

TGTAGTTCAG AAGAAGATTG GAGG

24

25

SEQ ID NO: 12

SEQUENCE LENGTH: 24

30

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

35

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

40

ATAATGGATC GCTTGGCAG TGAC

24

45

SEQ ID NO: 13

SEQUENCE LENGTH: 26

SEQUENCE TYPE: nucleic acid

50

STRANDEDNESS: single

TOPOLOGY: linear

55

MOLECULE TYPE: ther nucl ic acid, synthetic DNA

SEQUENCE DESCRIPTION:

TGAAGGTCGG AGTCAACGGA TTTGGT

26

SEQ ID NO: 14

SEQUENCE LENGTH: 24

SEQUENCE TYPE: nucleic acid

STRANDEDNESSS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

CATGTGGGCC ATGAGGTCCA CCAC

24

SEQ ID NO: 15

SEQUENCE LENGTH: 27

SEQUENCE TYPE: nucleic acid

STRANDEDNESSS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

CCATCCTAAT ACGACTCACT ATAGGGC

27

SEQ ID NO: 16

SEQUENCE LENGTH: 24

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

5 TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

10 GGATTTTCCT GATACTGTTT GTGG 24

15 SEQ ID NO: 17

SEQUENCE LENGTH: 24

SEQUENCE TYPE: nucleic acid

20 STRANDEDNESS: single

TOPOLOGY: linear

25 MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

30 ACCACAAACA GTATCAGGAA AATC 24

SEQ ID NO: 18

35 SEQUENCE LENGTH: 24

SEQUENCE TYPE: nucleic acid

40 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

45 SEQUENCE DESCRIPTION:

ACCTTTAAAA AGTCAACTGT CCTG 24

50 SEQ ID NO: 19



SEQUENCE LENGTH: 24

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

AAAAATTATC AAAAGGAATT TTGG

24

SEQ ID NO: 20

SEQUENCE LENGTH: 25

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

TTACTCTTAG TGAAGAGACA AACGC

25

SEQ ID NO: 21

SEQUENCE LENGTH: 36

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

AGCTGCGGCC GCGGTCTGCG GCTTAGCTCA AAATGC

36

SEQ ID NO: 22

SEQUENCE LENGTH: 36

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

AGCTGCGGCC GCAAAACAAA GAAAAGGAAA TCTGGT

36

SEQ ID NO: 23

SEQUENCE LENGTH: 37

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

AGCTGCGGCC GCAAGTGATG CCACCAAAA GAAATGA

37

SEQ ID NO: 24

SEQUENCE LENGTH: 36

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

AGCTGCGGCC GCTGGAAGGA AACACTGAAA TAAGAA

36

SEQ ID NO: 25:

SEQUENCE LENGTH: 10

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULE TYPE: protein

SEQUENCE DESCRIPTION:

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu

1 5 10

SEQ ID NO: 26

SEQUENCE LENGTH: 33

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

GATCGATCCC GTCTGCGGCT TAGGTCAAAA TGC

33

SEQ ID NO: 26

SEQUENCE LENGTH: 60

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

GATGGATCCT TAGAGGTCTT CTTCTGAGAT GAGCTTCTGC TCCTTCTGGA CTCTCTTTCT 60

SEQ ID NO: 28

SEQUENCE LENGTH: 33

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

GATGGATCCA GTGATGCCAC CAAAAGAAA TGA 33

SEQ ID NO: 29

SEQUENCE LENGTH: 60

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

GATGGATCCT TAGAGGTCTT CTTCTGAGAT GAGCTTCTGC TCGAGAAAAA ATAAAGCAAG 60

SEQ ID NO: 30

SEQUENCE LENGTH: 31

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

GATGGATCCC CATGCCTCGT GTAAAAGCAG C

31

SEQ ID NO: 31

SEQUENCE LENGTH: 31

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

GATGGATCCC CCAAAGAAAA GCAAATCTGG T

31

SEQ ID NO: 32

SEQUENCE LENGTH: 31

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

GATGGATCCC CATGCCACCA AAAAGAATG A

31

SEQ ID NO: 33

SEQUENCE LENGTH: 31

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

GATGGATCCC CACAACATTT CGGTGAAGAG C

31

SEQ ID NO: 34

SEQUENCE LENGTH: 29

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

CCTTGTGTTG TATGGGTGGA ACCCAGTGA

29

SEQ ID NO: 35

SEQUENCE LENGTH: 31

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

ACACCACGAT GCCTGGAGCA ATGGCAACAA C

31

5

SEQ ID NO: 36:

SEQUENCE LENGTH: 25

10

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

15

MOLECULE TYPE: protein

SEQUENCE DESCRIPTION:

20

Met Leu Pro Glu Ser Glu Asp Glu Glu Ser Tyr Asp Thr Glu Ser Glu Phe Thr

1

5

10

15

Glu Phe Thr Glu Asp Glu Leu

25

20

25

30

SEQ ID NO: 37:

SEQUENCE LENGTH: 19

SEQUENCE TYPE: amino acid

35

TOPOLOGY: linear

MOLECULE TYPE: protein

40

SEQUENCE DESCRIPTION:

Cys Gln Thr Glu Glu Ala Ile Gln Thr Arg Ser Arg Thr Arg Lys Arg Val Gln

45

1

5

10

15

Lys

50

## Claims

1. A protein having the amino acid sequence of SEQ ID NO: 2, or a protein having the same amino acid sequence where one or more amino acids are added, deleted, or substituted and having serine-threonine kinase activity.
2. A protein having the amino acid sequence of SEQ ID NO: 4, or a protein having the same amino acid sequence where one or more amino acids are added, deleted, or substituted and having serine-threonine kinase activity.



3. A protein encoded by a DNA sequence that hybridizes with the DNA sequence of SEQ ID NO: 1 or its complementary sequence and having serine-threonine kinase activity.
- 5 4. A protein encoded by a DNA sequence that hybridizes with the DNA sequence of SEQ ID NO: 3 or its complementary sequence and having serine-threonine kinase activity.
5. A DNA encoding the protein of any one of claims 1 to 4.
6. A vector comprising the DNA of claim 5.
- 10 7. A transformant carrying the vector of claim 6.
8. A method of producing the protein of any one of claims 1 to 4, wherein the method comprises cultivating the transformant of claim 7.
- 15 9. An antibody binding to the protein of any one of claims 1 to 4.
10. An antisense DNA against the DNA of claim 5 or part of it.
- 20 11. A method of screening compounds having inhibitory activity of serine-threonine kinase activity of the protein of any one of claims 1 to 4, wherein the method comprises
  - (a) contacting the protein of any one of claims 1 to 4 with a substrate to be phosphorylated by this protein in the presence of a test compound to detect the kinase activity of the protein of any one of claims 1 to 4, and
  - 25 (b) comparing the kinase activity detected in step (a) with that detected in the absence of the test compound and selecting a compound that lowers the kinase activity of the protein of any one of claims 1 to 4.



Fig. 2

A.

Consensus sequence of the active site of  
serine-threonine kinase

[Leu, Ile, Val, Met, Phe, Tyr, Cys]-Xaa-[His, Tyr]-Xaa-Asp-[Leu, Ile, Val,  
Met, Phe, Tyr]-Lys-Xaa-Xaa-Asn-[Leu, Ile, Val, Met, Phe, Tyr, Cys]-[Leu,  
Ile, Val, Met, Phe, Tyr, Cys]-[Leu, Ile, Val, Met, Phe, Tyr, Cys]

B.

Consensus sequence of the ATP binding site of protein kinase

[Leu, Ile, Val]-Gly-Xaa-Gly-Xaa-[Phe, Tyr, Trp, Met, Gly, Ser, Thr, Asn, His]  
-[Ser, Gly, Ala]-Xaa-[Leu, Ile, Val, Cys, Ala, Thr]-Xaa-Xaa-[Gly, Ser, Thr,  
Ala, Cys, Leu, Ile, Val, Met, Phe, Tyr]-Xaa(5 times or 18 times)-[Leu, Ile,  
Val, Met, Phe, Tyr, Trp, Cys, Ser, Thr, Ala, Arg]-[Ala, Ile, Val, Pro]-[Leu, Ile,  
Val, Met, Phe, Ala, Gly, Cys, Lys, Arg]-Lys

Fig. 3

```

      9      18      27      36      45      54
5' ACC TGG GTG TTC CTA AGT ATA GGG GGT CTG GTC TAC ATG ACA AAA ATG GAA AAA
-----
    T  W  V  F  L  S  I  G  G  L  V  Y  M  T  K  M  E  K
    P  G  C  S  *  V  *  G  V  W  S  T  *  Q  K  W  K  K
    L  G  V  P  K  Y  R  G  S  G  L  H  D  K  N  G  K  S

      63      72      81      90      99      108
GTT ACA GGT TTA TGA TAA TGG ATC GCT TTG GGA GTG ACC TTC AGA AAA TAT ATG
-----
    V  T  G  L  *  *  W  I  A  L  G  V  T  F  R  K  Y  M
    L  Q  V  Y  D  N  G  S  L  W  E  *  P  S  E  N  I  *
    Y  R  F  M  I  M  D  R  F  G  S  D  L  Q  K  I  Y  E

      117      126      135      144      153      162
AAG CAA ATG CCA AAA GGT TTT CTC GGA AAA CTG TCT TGC AGC TAA GCT TAA GAA
-----
    K  Q  M  P  K  G  F  L  G  K  L  S  C  S  *  A  *  E
    S  K  C  Q  K  V  F  S  E  N  C  L  A  A  K  L  K  N
    A  N  A  K  R  F  S  R  K  T  V  L  Q  L  S  L  R  I

      171      180      189      198      207      216
TTC TGG ATA TTC TGG AAT ATA TTC ACG AGC ATG AGT ATG TGC ATG GAG ATA TCA
-----
    F  W  I  F  W  N  I  F  T  S  M  S  M  C  M  E  I  S
    S  G  Y  S  G  I  Y  S  R  A  *  V  C  A  W  R  Y  Q
    L  D  I  L  E  Y  I  H  E  H  E  Y  V  H  G  D  I  K

      225      234      243      252      261
AGG CCT CAA ATC TTC TTC TGA ACT ACA AGA ATC CTG ACC AGG TGT 3'
-----
    R  P  Q  I  F  F  *  T  T  R  I  L  T  R  C
    G  L  K  S  S  S  E  L  Q  E  S  *  P  G
    A  S  N  L  L  L  N  Y  K  N  P  D  Q  V

```

Fig. 4

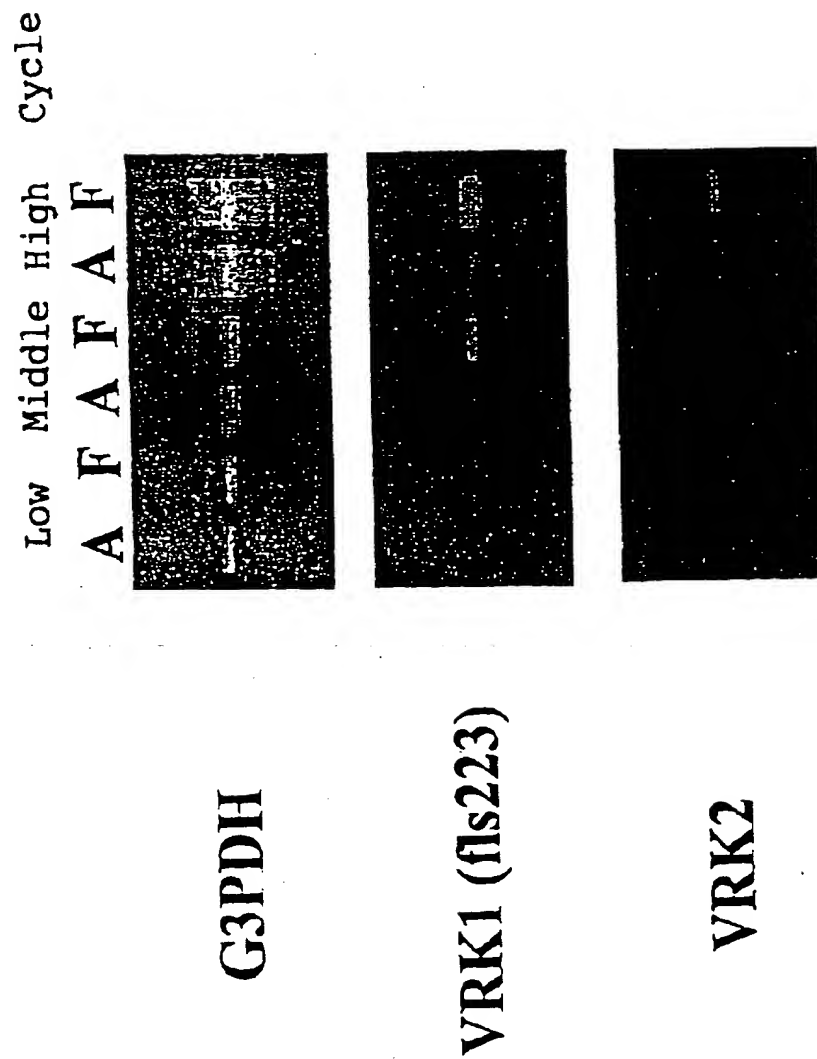


Fig. 5

	10	20	30	40	50	
VRK1	1 MPRVKAAG	RQSSAKRHLA	EQFAVGEIIT	DMAKKEWKVG	LPIGGGGFGC	50
B1R	1 M-----	-----	-NFQ-GLVLT	DNCKNQWVVG	PLICKGGGFGS	50
	60	70	80	90	100	
VRK1	51 IYLADMNSSE	SVGSDAPCVV	KVERSDNCPL	FTELKEYORA	AKPEQIQKWI	100
B1R	51 IY-----	-TTNDNNYVV	KIEPKANGSL	FTEQAFYTRY	LKPSVIEEWK	100
	110	120	130	140	150	
VRK1	101 RTRKLKYLGV	PKYWGSGLHD	KNKGSYRFMI	MDRFGSDLQK	IYEANAKRFS	150
B1R	101 KSHNIKHVCL	ITCKAFGLYK	SINVEYBELV	INRLGADLDA	VIRANNRRLP	150
	160	170	180	190	200	
VRK1	151 RKTVLQSLR	IEDILEYIHE	HEYVHGDIKA	SNLLINYKNP	DQVYLYDYGL	200
B1R	151 KRSYMLIGIE	ILNTIQFMHE	QCYSHGDIKA	SNIVLDQIDK	NKLYLYVDYGL	200
	210	220	230	240	250	
VRK1	201 AYRYCPEGVH	KEYKEDEKRC	HDCTIEETST	DAHNGVAPSR	RGDEHILGYC	250
B1R	201 VSKFMSNGEH	VPFIRNPKNM	DNGTLEETPT	DSHKGYYVSR	RGDLLETLYC	250
	260	270	280	290	300	
VRK1	251 MIQWLTGHLF	WED--NLKDP	KYVRDSKIRY	RENIASLMDK	CFPEKNKRGF	300
B1R	251 MIRWUGGILP	YTKISETKNC	ALYSATKOKY	VNNTATELMT	SL--QYARRE	300
	310	320	330	340	350	
VRK1	301 IAKYMETYKL	LDYTEKPLYE	NLRDYLQGL	KAIGSKDDGK	LDLSYVENG	350
B1R	301 LLOQITMVNS	LTYFEENYD	EFRIHLMQG	-----	-----	350
	360	370	380	390	400	
VRK1	351 LKAKTITKKR	KKEIEESKEP	GVEDTEWSNT	QTEEAIQTRS	RTRKNVQK..	400
B1R	351 -----	-----	-----	-----	---YYY..	400

Fig. 6

	10	20	30	40	50	
VRK1	1	MPRVKAAQAG	RQSSAKRHIA	EQPAVGEIIT	DMAKKEWKVG	LPIGGGGFGC 50
VRK2	1	MPP-----	-KRNEKYKLP	IPPEEGKVLD	DMEGNQWVLO	KKIGSGGEGC 50
	60	70	80	90	100	
VRK1	51	IYLAADMNSSE	SVGSDAPCVV	KVEPSDNGPL	FTELKEYQRA	AKPEQIQKVI 100
VRK2	51	IYLA--FPTN	KPEKDARHVV	KVEYQENGPL	FSELKEYQRA	AKKDCIKKVI 100
	110	120	130	140	150	
VRK1	101	RTRKLKYLGV	PKYWGSGLHD	KNCKSYRPMI	MDRFGSDLOK	FYEANAKRFS 150
VRK2	101	ERKQDYLGI	PLFYGSGLTE	FKGRSYRPMV	MERLGIDLAK	ISGQNGT-EK 150
	160	170	180	190	200	
VRK1	151	RKTVLQLSLR	IEDILEYIHS	HEYVHGDIKA	SNELLYKNP	DQVYLVDPGE 200
VRK2	151	KSTVLOLGIR	MLDVLEYIHS	NEYVHGDIKA	ANLLCYKNP	DQVYLADYGL 200
	210	220	230	240	250	
VRK1	201	AYRYCPGVH	KEYKEDRKRC	HDGTIEETSI	DAHNGVAPSR	RGDLETLYG 250
VRK2	201	SYRYCPGVH	KQYQENERKG	HNGTIEETSL	DAHNGVALSR	RSBVELEGY 250
	260	270	280	290	300	
VRK1	251	MIQWLTGHL	WEDNEKDPKY	VRDSKIRYRE	NIASLMDKCF	PEKNKPGEIA 300
VRK2	251	MLRWLCGKL	WEONLKDPVA	VQTAKTNLLD	ELPQSVLRWA	ESGSSCCETA 300
	310	320	330	340	350	
VRK1	301	KYMETVKLLD	YTEKBLVENE	RDIELQGLKA	IGSKDDGKLD	LSV----- 350
VRK2	301	QFLVCAHSLA	YDEKPNQAL	KKLENPHGIP	LGPLDFSTKG	QSINVHTPNS 350
	360	370	380	390	400	
VRK1	351	--VENGGLKA	KTITKRRKKE	TEES--KEPG	VED-TEKSNT	QTE----- 400
VRK2	351	QKYDSQKAAT	KQVNAHNRL	TEKKVHSERS	AESCATEKVQ	KEEKLIGLMN 400
	410	420	430	440	450	
VRK1	401	-EAIQTRSNT	RKRVRK----	-----	-----	----- 450
VRK2	401	NEAAGESTER	RQKYQESQEP	LNEVNSFPQK	ISYTQFPNSF	YEPHQDFTSP 450
	460	470	480	490	500	
VRK1	451	-----	-----	-----	-----	----- 500
VRK2	451	DIFKKSRSPS	WYKYTSTVST	GITDLESSTG	LWPTISQFTL	SEETNADVYY 500
	510	520	530	540	550	
VRK1	501	-----	-----	-----	-----	----- 550
VRK2	501	YRIIPVLLM	LVFLALFFL	-----	-----	----- 550

Fig. 7

		10	20	30	40	50	
VRK2	1	MPPKRNEKYK	LPIPFPEGKV	LODMEGNQV	LGKKIGSGGF	GLIYLAFPTN	50
B1R	1	MN-----	E-QGLV	ETDNCKNQV	VGPLIGKGGF	GSILY-----	50
		60	70	80	90	100	
VRK2	51	KPEKDARKVY	KVBYQENGPI	ESELKPYQRY	AKKDCIKKWI	ERKQLDYLGI	100
B1R	51	-TTNDNNYVY	KLEPKANGSI	FTEQAEYTRV	LKPSVIEERK	KSHNIKHVGL	100
		110	120	130	140	150	
VRK2	101	PLFYGSQETE	FKGRSYRPMV	MEREGIDLQK	-ESGQNGTFK	KSTVLQLGIR	150
B1R	101	ITCKAFGUYK	SINVEYRFLY	INRLGADLDA	VIRANNRLP	KRSYMLIGIE	150
		160	170	180	190	200	
VRK2	151	MEVDLEYIHE	NEYVHGQVKA	ANLLEGYKNP	DQVYIADYGE	SYRYCPNQNH	200
B1R	151	ILNTIQPMHE	QGYSHGDIKA	SNIVLDQIDK	NKLYLVVDYGE	VSKFMSNGEH	200
		210	220	230	240	250	
VRK2	201	KQYQENRRKG	HNGTIEETSL	DAHKGVALSE	RSEVIELEGY	MLRWLCGKEE	250
B1R	201	VPFIRVNNKM	DNGTLEETPI	DSHKGYYVSS	EGELETLGCG	NIRWLCGQIR	250
		260	270	280	290	300	
VRK2	251	WEQ--NLKDP	VAVQTAKTNL	LDE---LPQS	VEKWAESGSS	CCBIAQFLVC	300
B1R	251	ETKISETKNC	ALYSATQOKY	VNNTATELMT	SEQYABR---	ELLOYITM	300
		310	320	330	340	350	
VRK2	301	AHSCAYDEKP	NYQALKKIEH	PHGIPLGPLD	FSTKGQSINV	HTPNSQKVDS	350
B1R	301	VNSETYFEEF	NYDEFRIEEM	Q-----	-----	-----	350
		360	370	380	390	400	
VRK2	351	QKAATKQVNK	AHNRLIEKKV	HSERSAESCA	TWKVQKEEKL	IGLMNNEAAQ	400
B1R	351	-----	-----	-----	-----	-----	400
		410	420	430	440	450	
VRK2	401	ESTRRRQKYQ	ESQEPLNEVN	SFPQKISYTD	FPNSFYEPHQ	DFTSPDIFKK	450
B1R	401	-----	-----	-----	-----	-----	450
		460	470	480	490	500	
VRK2	451	SRSPSWYKYT	STVSTGITDL	ESSTGLWPTI	SQFTLSEETN	ADVAYYRIII	500
B1R	451	-----	-----	-----	-----	-GYH-----	500
		510	520	530	540	550	
VRK2	501	PVLLMLVFLA	LFFL.....	.....	.....	.....	550
B1R	501	-----	-----	-----	-----	-----	550



Fig. 8

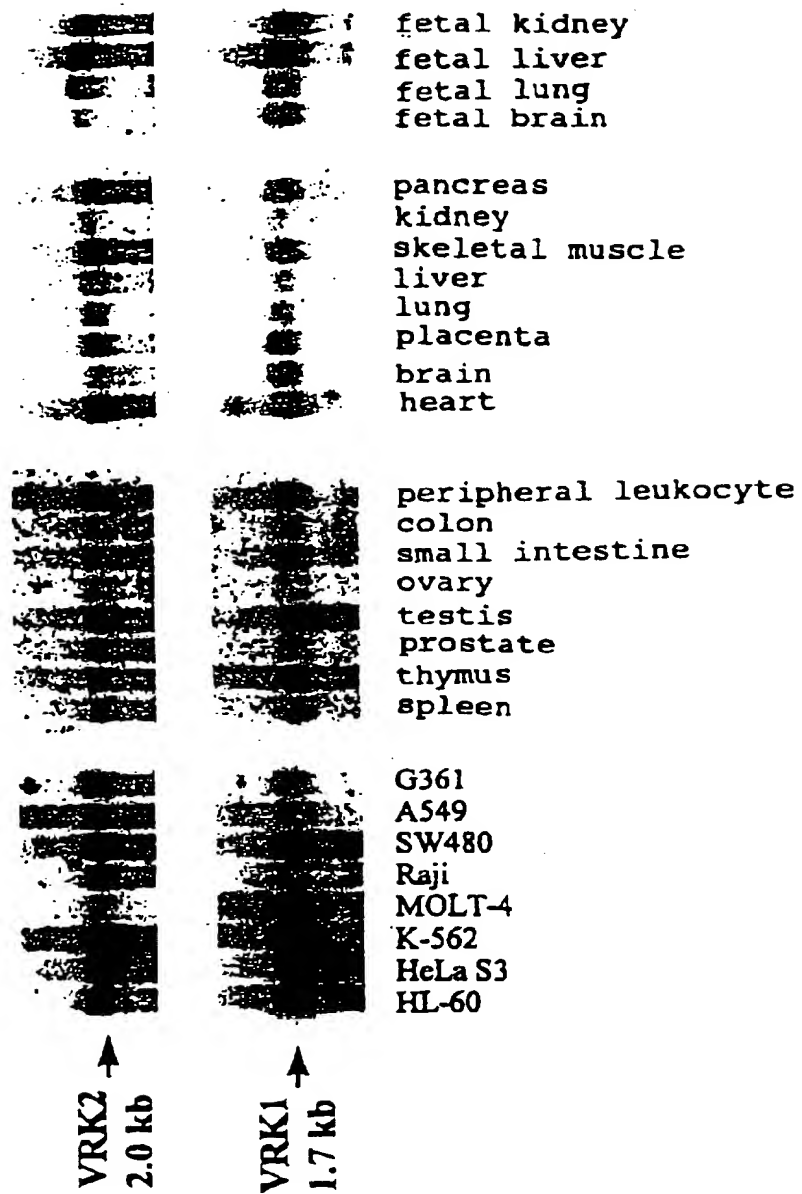


Fig. 9

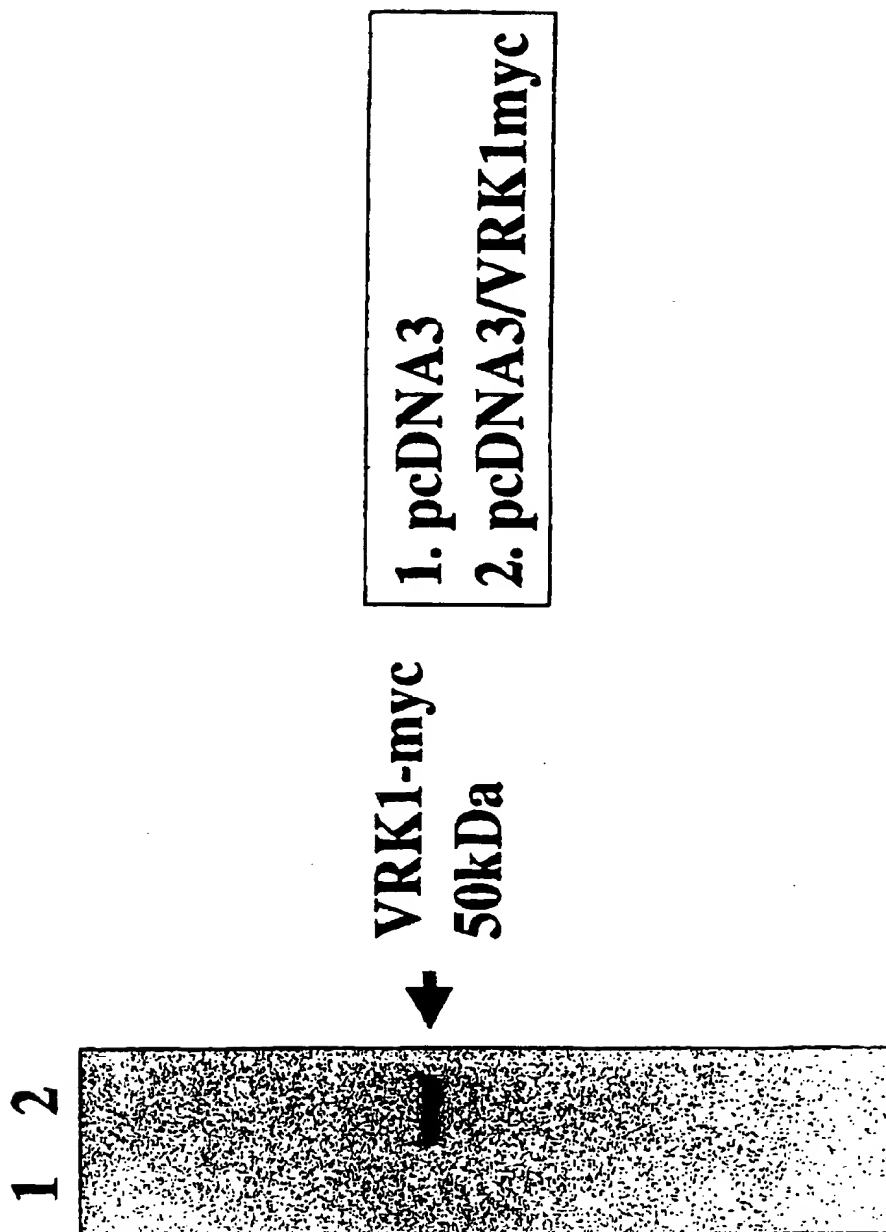


Fig. 10

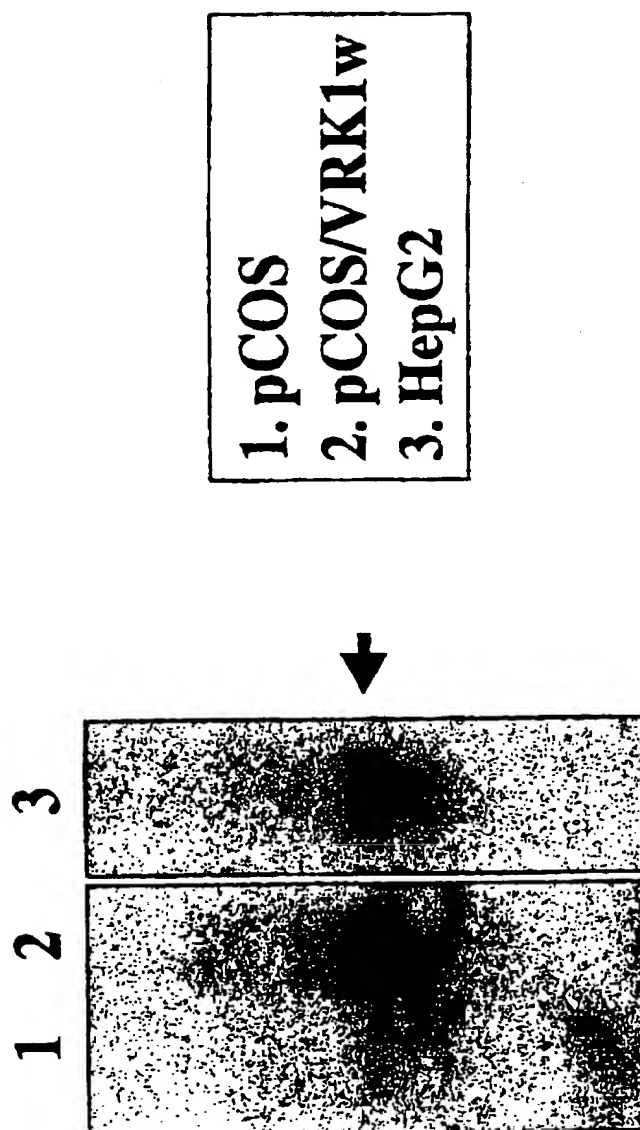
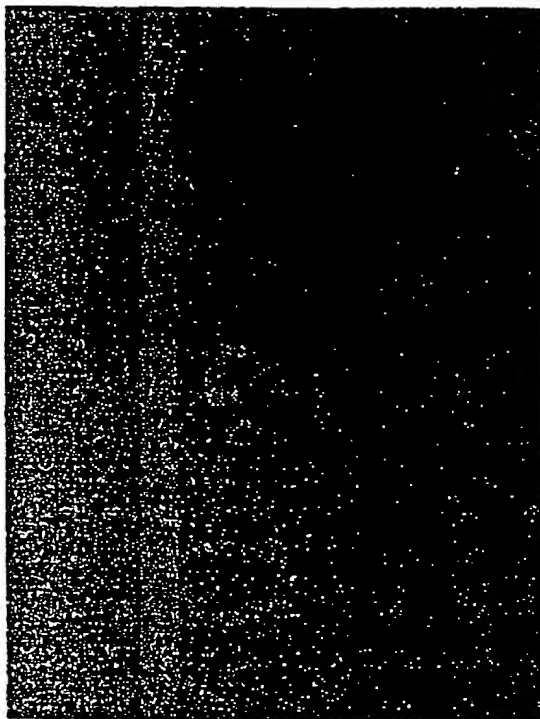


Fig. 11

pCOS/VRK1w



pCOS

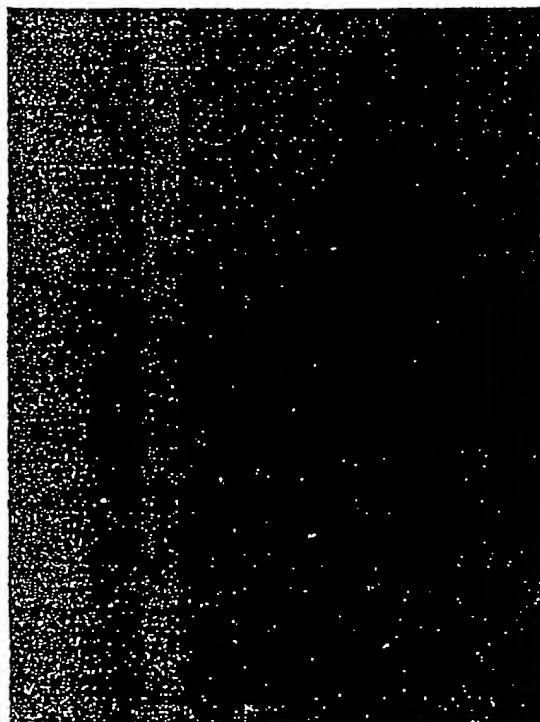


Fig. 12

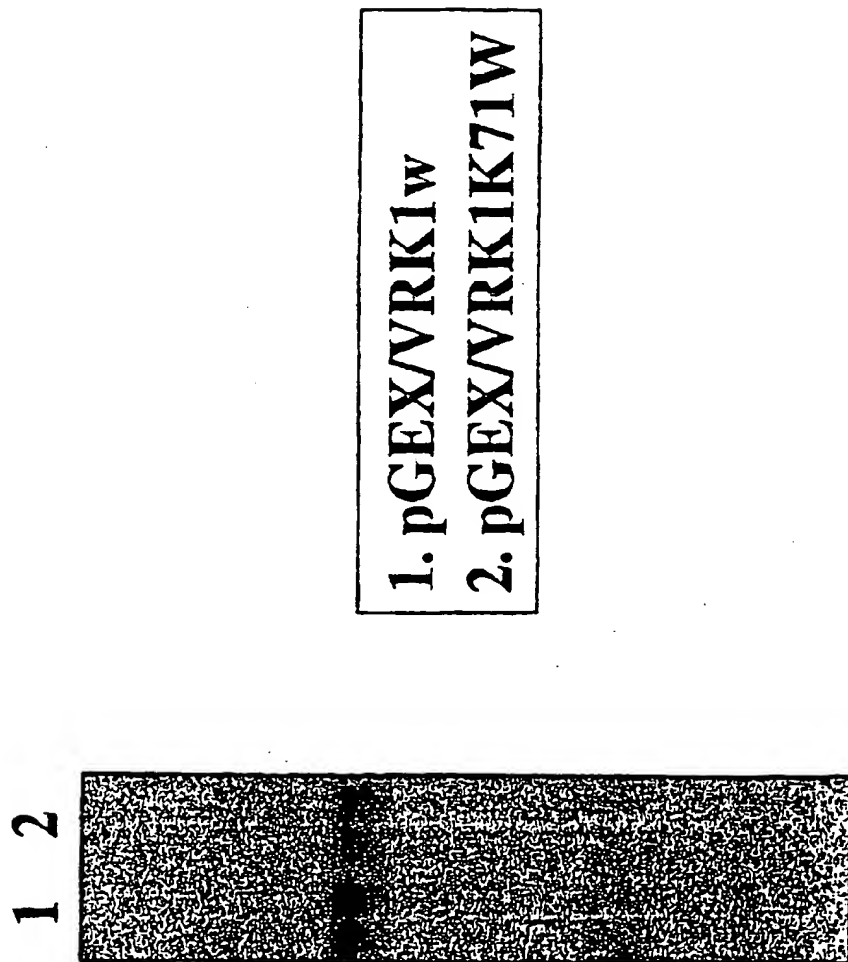


Fig. 13

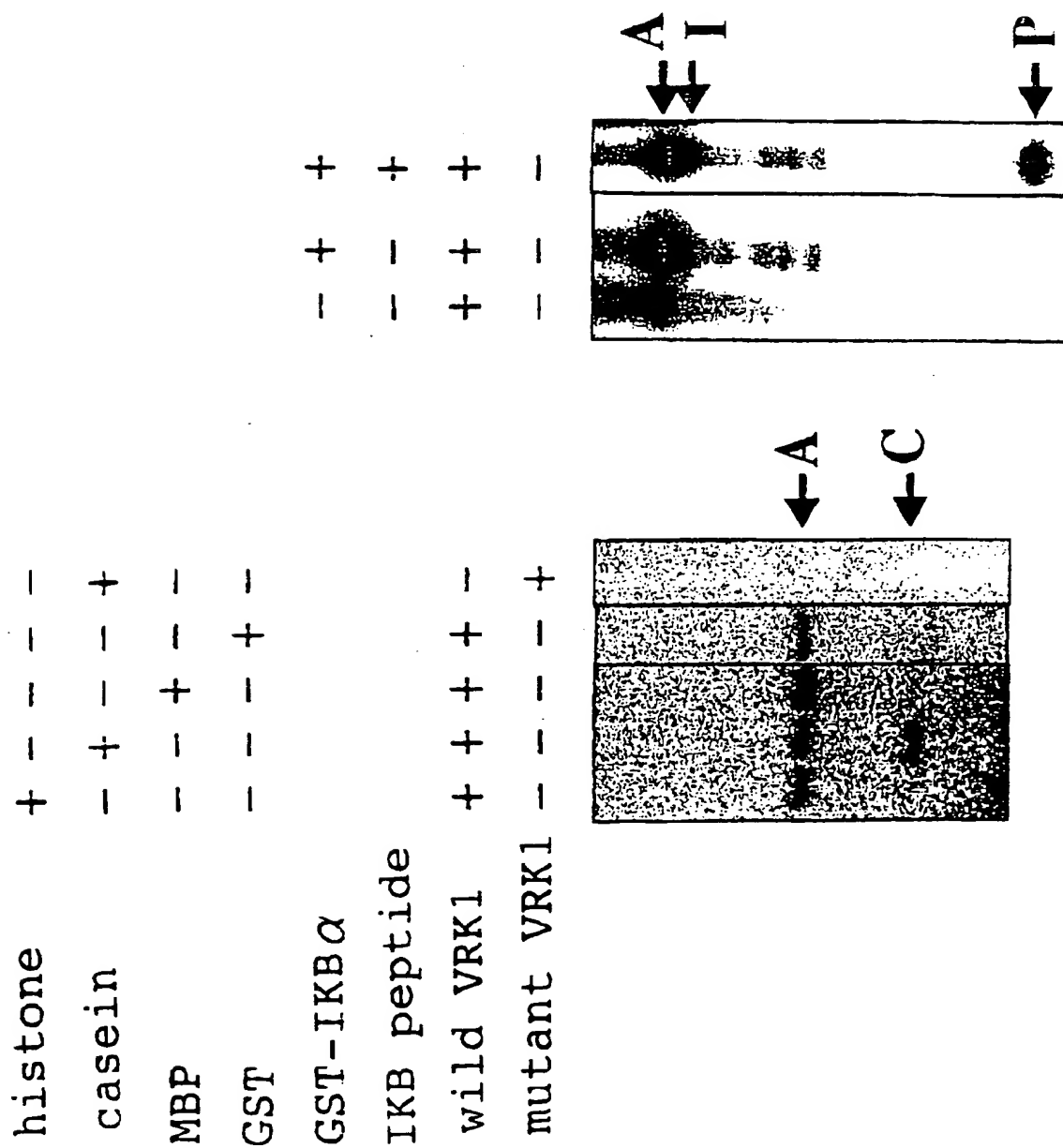


Fig. 14

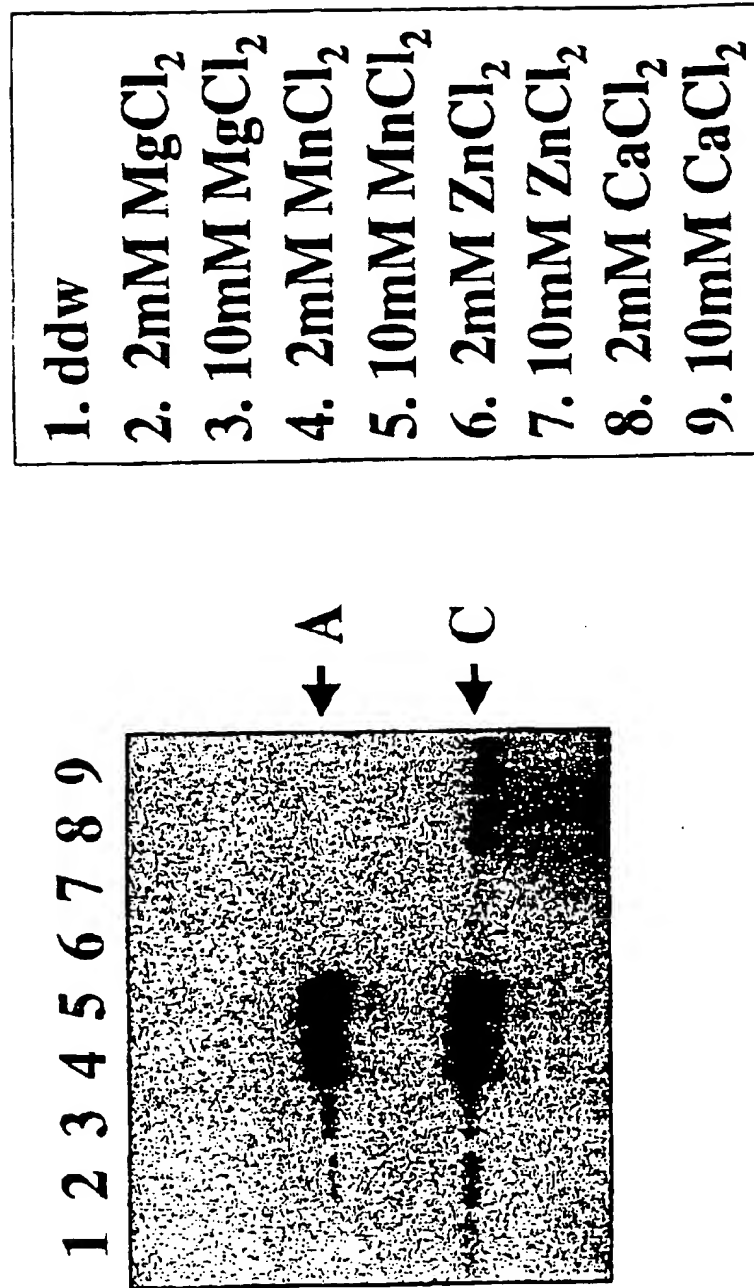
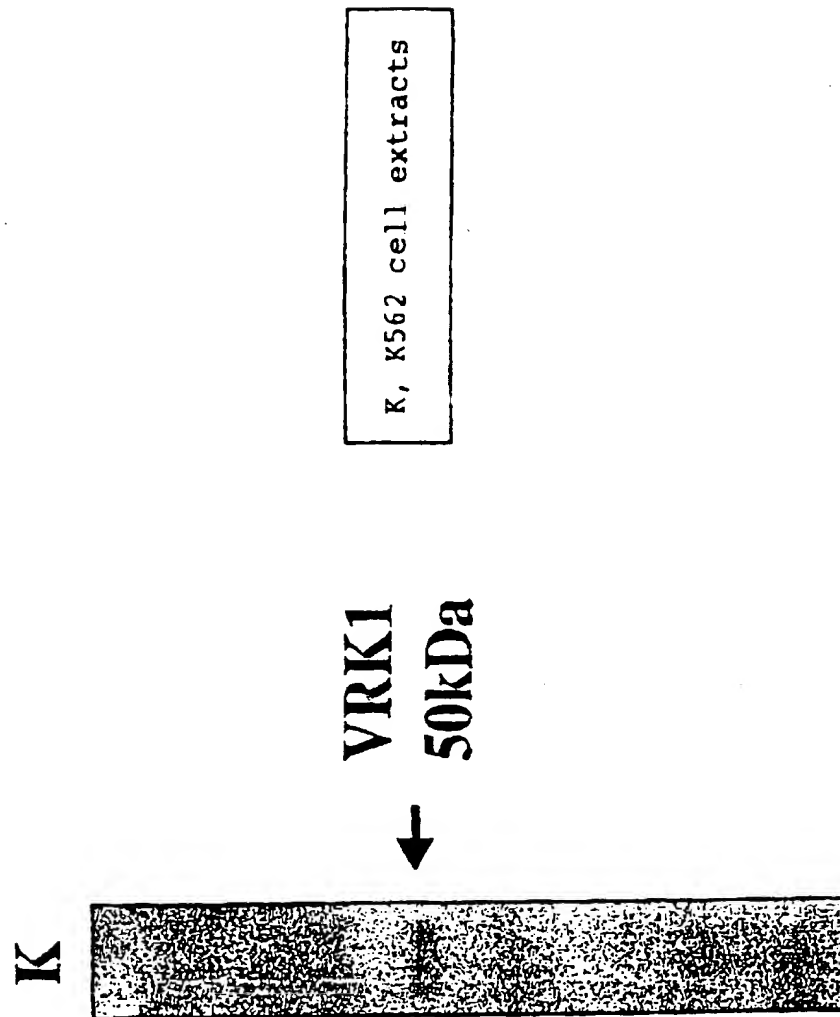


Fig. 15





## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP97/04855

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
Int. Cl <sup>6</sup> C12N15/54, C12N5/00, C12P21/02, C12Q1/48, C12N9/12		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols)		
Int. Cl <sup>6</sup> C12N15/54, C12N5/00, C12P21/02, C12Q1/48, C12N9/12		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
Genbank/EMBL/DDBJ (GNEETYX), BIOSYS (DIALOG), MEDLINE (STN)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	Genomics, Vol. 45(2) (1997-Oct) Nezu J-I et al. "Identification of two novel human putative serine-threonine kinases, VRK1 and VRK2, with structural similarity to vaccinia virus B1R kinase" p. 327-331	1 - 11
A	Proc. Natl. Acad. Sci. USA, Vol. 92(18) (1995) Lee N.H. et al. "Comparative expressed-sequence-tag analysis of differential gene expression profiles in PC-12 cells before and after nerve growth factor treatment" p. 8303-8307	1 - 11
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "A" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
February 2, 1998 (02. 02. 98)		February 10, 1998 (10. 02. 98)
Name and mailing address of the ISA/ Japan Patent Office		Authorized officer
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